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The haustoria of certain rusts and the relation between host and pathogene

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The haustoria of certain rusts and the relation between host and pathogene[1]

MABEL AGNES RICE

(WITH PLATES 1–9)

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INTRODUCTION

The relations of host and parasite in the case of plants infected by the higher fungi whose structure is so highly differentiated afford a specialized field for the study of fundamental pathogenic relations. The following study of haustoria has been undertaken with the aim of contributing toward the solution of the problems of susceptibility and immunity and the relations between the higher parasitic fungi and their hosts.

Probably the earliest mention of haustoria is that of Visiani of the Venetian Commission which reported upon the grape mildew in 1851. Von Mohl (1863) reports that Visiani claimed

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that 'Haft-organe' upon the surface of the leaf send penetrations 'nach Art von Wurzeln in das Gewebe der Epidermis.' Von Mohl discredits the idea of cell penetrations and figures only 'Haft-organe' for the grape mildew. DeBary (1863) was the first to describe adequately the occurrence and structure of haustoria. He apparently first used the term haustorium for the knob-like organs of *Cystopus*. He described and figured many examples of haustoria in the Peronosporeae and in the Erysipheae. Through DeBary's studies in the Erysipheae, Visiani is confirmed. In Vergleichende Morphologie und Biologie der Pilze (1884) DeBary reproduced Von Mohl's figure of *Erysiphe Tuckeri*, which shows 'Haft-organe' only, and on the same page figured attachment disk and haustorium for *Podosphaera Castagnei*. He included the attachment disk as a typical part of the structure, describing the haustoria as 'Haft- und Saug-organe.' He correctly regarded the haustorium as a specialized organ that maintains the interrelations between parasite and host. The most careful of the modern studies upon haustoria has been made in the group of the Erysipheae by Grant Smith (1900).

Work upon haustoria in the Uredineae seems to have developed more slowly than in the other groups of fungi. In 1863 DeBary stated that he had first thought haustoria in the Uredineae were limited to perennial species but that he had found them also in non-perennials. In 1867 he described the branched and knotted haustoria of *Aecidium elatinum*. Somewhat vague drawings of haustoria for both *Puccinia Malvacearum* and *Uredo graminis* were given by Carlo Bagnis (1875). Ward (1882) was the first to present detailed work upon haustoria of the Uredineae, when he described and figured the haustoria of *Hemileia vastatrix*. Ward's later work (1902, 1904) upon *Uredo dispersa* in the brome grasses centered the problems of parasitism in the Uredineae upon the haustorium.

The bearing of the haustorial relation between host and pathogene upon the nature, origin, and development of parasitism has been but little developed since Ward's introduction. Many simple and obvious questions as to the structure and function of haustoria cannot be regarded as settled. Are they purely nutritive, and is the haustorium the most perfect and least destructive means by which a parasite can draw food from a host?

Does it, on the other hand, imply destructive attack by physical or by chemical means? Does it imply toxic action of the parasite on the host, or does the presence of haustoria imply high resistance in the host as compared with the ineffectual resistance of the host to *Pythium* or *Botrytis?* How does the penetration of the wall by a haustorium compare with the intracellular parasite in its relations to its host? Data upon such questions relate directly to important problems of susceptibility and immunity.

REVIEW OF LITERATURE

The evolutionary classification of parasitism in the fungi

The question of the origin and nature of parasitism itself underlies questions concerning haustoria. Have parasites developed from saprophytes? In the field of bacteriology, Oscar Bail argues for such an origin, and suggests that the search for food may have led a saprophyte into the bodies of living animals. Infection is defined by Bail (1911) as the colonization of an organism within the same space which another organism fills with its own functioning. He applies his theory in a classification of bacteria, which he calls an attempt at a natural system of infective organisms. On the basis of their invasive power he recognizes: first, the pure saprophytes which can under no conditions colonize within foreign organisms, the necro-parasites, like the tetanus bacillus, which enter living hosts through dead tissues and overcome the host cells by toxins; second, the half parasites of the typhus type, whose successful infection is limited to definite body regions, and whose poisons are bound to the bacterial body; finally, the pure parasites, which, with increased aggressiveness and power of infection, have little of toxicity: '. . . je besser sie das Infizieren gelernt haben, nicht ihren Nährboden früher zerstören, ehe sie ihn nicht überall ausgenützt haben.' The organisms producing septicemia he gives as an example of this third type.

Theobald Smith (1921) likewise develops the idea of an evolutionary series of bacteria, classified by the character of their invasive power. At one end of the scale, he places types just emerging from the predatory or saprophytic stage and acquiring parasitic habits; at the other end, the highly specialized forms adapted to one host, species, or race. Agents of septic infection

illustrate the saprophytic predatory type. Next he places the semi-saprophytic diphtheria bacillus, which offers a direct offensive to the host body by toxins that attack and injure living tissues. (These are Bail's necro-parasites.) Then he notes an adapted type where the offensive devices of invasion are under control, such as the tubercle bacillus with which the host frequently establishes a balanced life. Finally, he notes the development of the highly specialized parasite, such as the smallpox organism, which 'depends upon one host for its existence' and has established such a balance with that host as to be able to complete a full life cycle of invasion, multiplication, and dissemination. There are, Smith adds, no obstacles to the activity of these organisms except immunity.

DeBary (1884) turned the attention of mycologists to the physiological viewpoint by his painstaking work upon structure and function in the fungi. He was apparently the first mycologist to attempt an evolutionary classification of the fungi on a physiological basis. Unlike the bacteriologists with their emphasis upon invasive power, he based his classification on degrees of adaptation in obtaining nutrition. He distinguished: first, pure saprophytes; second, the facultative parasites, species normally saprophytes but with the power of going through their development wholly or in part as parasites; third, the facultative saprophytes, which as a rule go through the whole course of their development as parasites (indeed a parasitic life is indispensable to the attainment of their full development) but which are able in certain stages to vegetate as saprophytes; finally, the obligate parasites, species which live only as parasites.

Van Tieghem (1873) used the term facultative parasitism in connection with *Chaetocladium*, *Piptocephalis*, and *Syncephalis*, to indicate a parasitism 'avantageux sans doute, mais non necessaire.' In explanation he refers to several degrees of parasitism leading to one which implies 'une condition absolue d'existence,' but DeBary was apparently the first to balance the term obligate against facultative. DeBary (1884) removed *Piptocephalis*, *Syncephalis*, and *Chaetocladium*, the group for which Van Tieghem coined the phrase facultative parasites, to the group of facultative saprophytes on the ground that they form zygospores only when they live as parasites.

DeBary places the rusts together with the lichen fungi at the

head of the obligate parasites. This linking is made upon the basis of the limited host accommodation on the part of both rusts and lichen fungi, the character by which DeBary recognizes the extreme of obligate parasitism. In discussing the effects on the host and the reactions of the host, he separates the rusts and the lichen fungi. He places many rusts, such as the species of *Puccinia* infecting grasses, in the group of destructive fungi. The other rusts he places in the group which cause in varying degree in their hosts 'mycetogenetic metamorphosis.' He places the lichens at the head of this group. In this, as well as in his main divisions, DeBary's emphasis is upon nutrition. He regards all these grades of parasitism as 'in direct causal connection with the process of feeding on the part of the fungus.' Raines (1922) goes a step further by tracing an interaction between obligateness and mutualism:

> In the group of the fungi the transition from violent and destructive parasitism to parasitism of the symbiotic type is accompanied by a transition from facultative to obligate parasitism as if the physiological corollary of parasitism of the latter type is extreme specialization in food preferences.

The real relation between these classifications of the bacteriologists and mycologists is in need of further study. In the classifications of bacteria quoted above, the basis is that of attacking power of the parasite, while for the mycologists the basis is that of method of nutrition. The two views are not inconsistent. Indeed the quotation from Raines indicates how functions of offence and of nutrition may interact. An understanding of both the method of nutrition and the change from the production of toxic products to the production of neutral products, so far as the host is concerned, is necessary in a full analysis of the interrelations of two cells achieving structural as well as functional adaptations toward symbiosis.

To a greater or less degree one finds presented in various handbooks on fungi the conception of an evolution from saprophytic to obligate parasitic types. Frank (1895) divides parasitic fungi into an obligate and a facultative group, making the possibility of artificial culture the characteristic of the facultative parasite, over against those which can be brought to a complete development only upon a living host. However, instead of treating the obligate as the climax of an evolution, he considers the method of attack of a facultative parasite the stronger, since

'the conditions of its entrance and development in this case are much broader.'

Massee (1899) regards parasitism as an acquired condition, and he traces gradations in injury to the host. He commences with *Pythium*, which 'lacking experience, kills its host plant within a very short period, thus limiting its own existence to a matter of hours.' (Here we have Smith's semi-saprophyte.) At the other extreme he places organisms that have evolved a method of living with the host plant with comparatively little injury to it—as for instance the smuts, which in many cases cause little or no injury during the vegetative period of the host; the parasite of *Lolium temulentum* which hibernates in the aleurone layer of the seed and lives in such complete mutualism as an intercellular parasite through the whole life cycle of the host that all spore production on the part of the fungus has been eliminated; and the cereal rusts, which attack only specific hosts and by means of haustoria live for long periods in intracellular relations with them.

Klebahn (1912) begins his evolutionary discussion with the spot fungi, which, he states, are parasitic in attack, but become saprophytes, causing death of the tissue by poison enzymes. He illustrates varying degrees of this toxic action as found in *Botrytis*, *Pythium*, *Phytophthora*, *Septoria*, and *Gleosporium*. Over against this class, which causes a more or less early death of the host tissue, stand, in Klebahn's list, those strict parasites which grow through the tissue and nourish themselves thereon without killing. Here the browning of tissue is less in evidence. There may be fading or yellowing or there may be the occurrence of new color (anthocyan). In this class of strict parasites Klebahn places the Uredineae, Ustilagineae, and Oömycetes. These groups, according to Klebahn's description, would rank with the adapted type of bacterial parasites for which Smith emphasizes immunity as the only obstacle.

Butler (1918) presents the most detailed physiological classification found in any of the handbooks. He traces the parasitic strain as one 'educated' from hemi-saprophytes, parasitic only occasionally on 'weak hosts,' through wound parasites, to those able to attack sound tissues. The true parasites he subdivides on the basis of variations in methods of feeding.

The references given indicate that DeBary's classification is generally recognized; but the relatively small space given the

discussion in the various handbooks indicates that a physiological classification of fungi in their relations to the substratum is still only in the stage of a working hypothesis. That it may be more than a working hypothesis in the future, Blackman (1924) forecasts as follows: 'We may anticipate a time when these hidden reactions (the physiological processes of the parasite) may be revealed and a new basis for the classification of plant diseases established.'

In this connection, the facultative parasites are of special interest since here one probably finds the parasitic habit in its beginning and one may contrast the evidence of toxic action by the invading hyphae of the non-haustorial facultative parasite with the evidence of fine adjustment to its host on the part of the obligate haustorial parasite. *Botrytis* and the related Peziza are instructive types of this facultatism. Indeed Ward (1890) states in his Croonian lecture that it is apparently possible to carry the process of 'educating' the *Botrytis* genus to habits of parasitism much further than in any other cases known. His description in this lecture of the behavior of a *Botrytis* illustrates the non-adapted habit of a fungus which succeeds in devouring its young soft host:

> So long as the protoplasm can dispose of small amounts of poison coming in from the hyphae, the hypha is debarred access to the cells but immediately the poison succeeds in lessening or destroying the power of the protoplasm to control the cell sap, the latter exudes through the permeable protoplasm and suffuses the whole tissue with the cell sap, containing just such food materials as the fungus flourishes in. The fungus can flourish while these products last; but the death of the host tissue results in the early end of the food supply.

The *Botrytis* hypha is here an instrument of destruction and is a very successful instrument of its kind. There is specialization in the case of the saprophyte, which consumes the whole body of the host at once, but the more successful type requires of its host continuous small quantities of food for a long period. This is the habit of an adapted parasite.

In *Peziza Sclerotiorum*, DeBary (1884) describes the beginnings of parasitism, for, although he cultured the fungus as a saprophyte on a variety of media, he proved that it would attack healthy tissue also, provided that the spores were first germinated under saprophytic conditions. He believed that penetration

into the host membrane is effected by aid of a ferment, which can be formed only by a properly nourished germ tube. DeBary was not able to obtain a conidial stage for his *Peziza*, but its relationship to *Botrytis* is generally accepted. Ward implies this in his comparison of DeBary's results and his own work with a lily-*Botrytis*. Of the latter he writes (1888): 'I am driven to conclude that although I have never yet succeeded in growing sclerotia or peziza-cups from the *Botrytis* it is either a stage in the life history of a *Peziza* of some kind or at least its alliance lies in this direction.' Of DeBary's fungus he writes: 'Accepting DeBary's results with *Peziza Sclerotiorum*, I regard his fungus as a form physiologically midway between the ordinary saprophytic Pezizas, and my parasitic one; in other words, DeBary's fungus is in process of being educated to parasitic habits.' Ward offers his lily-*Botrytis* as illustrating the next higher step in parasitism, because of its power of direct penetration into living tissue. This unusual instance of a *Botrytis* able to penetrate a living host unaided, he explains as due to the water-gorged state of the lily tissue caused by an excessively rainy season, but the variable power of the fungus, he believes, is shown by a differential behavior within the host tissue. He finds in the infected lily plants not only cases of the normal *Botrytis* saprophyte, where the fungus ferment kills the tissue, and the mycelium is trapped in the shrivelled mass of brown dead cells, but also cases where the invader, although destructive to adjacent cells, is able to advance to new areas through the water-soaked walls. Thus within one tissue one may see the shift from the predatory saprophyte to the half parasite. In addition to the added power of penetration into the soft host, the essential difference lies in a finer adjustment between invader and host, which brings death less quickly as the result of infection.

Ward concluded that his lily-fungus was 'a so-called *Botrytis* of the *Polyactis* type—distinct from *Polyactis vulgaris*, *Botrytis cinerea* and other accepted forms.' Later work adds evidence of the complexity of this type. Klebahn (1909), working with *Botrytis cinerea* on *Syringa*, *Pelargonium*, and *Adiantum*, concluded that 'under the name *Botrytis cinerea* many forms are assembled which have developed in varied degree the power to infect and are also in part more or less adapted to quite definite hosts.' He found a considerable degree of parasitism for the

species, along with some specialization in hosts. Conidia from *Syringa* leaves infected *Syringa* readily, but infected *Pelargonium* and *Adiantum* in decreasing degree. Conidia from *Pelargonium*, however, gave vigorous infection upon *Syringa*. In all cases, he stated, the infection occurred without any preliminary saprophytic nourishment, and without any artificial injury or killing of the host tissue. We have here a further advance in the facultative parasitism of *Botrytis*.

The connection between a *Botrytis* and a *Sclerotinia* has been established by Seaver and Horne (1917). They obtained the *Botrytis* form directly from the peziza cups of sclerotia on rhizomes of *Geranium maculatum*. The cultures were similar to the conidial stage, which was also abundant upon the *Geranium*. *Sclerotinia Geranii* appears from their description to be a specific parasite.

Brierly (1919) from the genetical standpoint supports the assumption of a multiple *Botrytis*. He is unable, he states, to 'educate' *Botrytis cinerea* unless the initial culture consists of a mixed population.

A further indication of specificity in the relation of *Botrytis* to different hosts is confirmed and illustrated by Hopkins (1921) in his study of *Botrytis Tulipae*. Not only was its parasitism demonstrated by numerous infections of tulips from pure cultures, but gradations in parasitism were shown by the weaker and varying ability of the pathogene to attack such other plants as narcissus, onion, crocus, and hyacinth.

The specificity in host relations in this case, however, does not necessarily imply an advance in adaptive parasitism. There may be here merely the preference of a fungus for one food over another; but from this tendency an omnivorous saprophyte may develop physiological species, and may even evolve into a parasite upon a favorable host. Thus the later studies upon *Botrytis* really corroborate the ideas of DeBary and Ward as to the evolution of parasitism. Both in the occasional finer adjustment between invader and host which brings death less quickly as the result of infection, and in tendencies toward specificity in hosts, this example of a facultative parasite suggests something of the manner of life of the haustorial fungus. However, it is only facultative parasitism at the most, and *Botrytis* in general illustrates what the obligate parasite with haustoria does not do.

This is emphasized by Ward (1890), who concludes his discussion of the relation between host and parasite by balancing against his description of *Botrytis* and its host relations a description of conditions among the Uredineae: 'In all cases where haustoria are developed, the mycelium enters into a peculiar symbiotic connection with the cells and for some time merely taxes them as it were rather than injures them directly.' Such phenomena, Ward believes, 'tend to the subject of symbiosis proper where relations between host and parasite have become so arranged that both may be said to benefit by the commensalism.'

Thomas (1921) also brings out the same contrast in parasitic habits when he outlines an intergrading series of parasites according to completeness of adaptation to the host. Commencing with *Botrytis*, which kills in advance, he points to the increasing tendency among various parasites to show features of mutualism and symbiosis as adaptation becomes more nearly complete. He regards the seed fungus of *Lolium temulentum* as a climax form, since 'it is perpetuated entirely as a mycelium through the seed of the host and perhaps never kills any of the host cells.'

The tendency of parasites to increase reproduction at the expense of vegetative growth and differentiation is almost universal. The group of the Rhizoctonias and *Lolium* are, however, marked exceptions, which seem to perpetuate themselves entirely, or largely, by mycelium or sclerotia. *Corticium vagum Solani*, the basidial stage demonstrated by Rolfs (1904) for the *Rhizoctonia* on the potato, does not seem necessary for perpetuation of the fungus. Duggar and Stewart (1901) and Rolfs (1904) think that *Rhizoctonia* may live indefinitely as a soil saprophyte and infect the potato under conditions unfavorable to host growth. Whether the seed fungus and the sterile Rhizoctonias represent the highest degree of specialization physiologically is a question dependent upon how the suppression of spore production is to be interpreted. Vegetative growth may itself be a drain upon a host. The thread blight of tea, a possible *Rhizoctonia* described by Butler (1918), produces no spores but causes a browning of the tea leaf under the heavy web of mycelium. *Corticium vagum Solani* is at any rate proved a more specialized parasite than its *Rhizoctonia* stage. Rolfs states of the *Corticium* stage: 'This stage has been observed only on or near green potato plants. . . . All attempts in the laboratory to de-

velop spores on various culture media have failed.' Peltier (1916), who has found the *Corticium* stage on a number of garden plants, observes: 'This stage is generally found on plant tissues that are perfectly healthy; it is in no way injurious to them.'

The rusts, in contrast to these characterless organisms which have apparently lost in differentiation, show a high degree of specificity in spore forms.

Relations between host cells and intracellular parasites

Two types of intracellular fungi are familiar: first, the common case in which mycelial hyphae merely pierce and grow through the host cells, as do *Pythium* and *Rhizopus;* second, the case in which the hyphae form special coils or wefts in the host cells, as do the fungi of certain Mycorrhizas, or live entirely within the host cell, as do certain Chytridiaceae.

Piercing host cells by haustorial processes seems to be a very different thing from piercing host cells by entering hyphae, as in the case of *Pythium.* Higgins (1914) describes a *Septoria* whose hyphae pass directly through cells of *Prunus pennsylvanica,* killing the leaf tissue outright. Stevens and Hawkins (1917) show that *Rhizopus* causes 'leak' in the strawberry by piercing the cells and killing the protoplasts. Hawkins and Harvey (1919) claim that *Pythium* in the potato tuber punctures by pressure, and then kills the invaded cells by toxins.

The haustorial penetration shows analogies to the behavior of the unicellular, intracellular, parasitic fungi. The method of penetrating the host cell and the interrelations of the protoplasm of host and parasite in these cases should throw light on the nature of haustoria and their functions. The haustorium is, at least so far as the cell wall is concerned, an intracellular portion of a parasite which is otherwise intercellular with respect to its host. So far as we know haustoria are primarily absorption organs, although specific evidence as to what and how they absorb is as yet lacking. They seem to effect an increased contact area between host and fungus, approximating that maximum contact attained by such intracellular parasites as the tubercle organisms of legumes, the parasitic Myxomycetes, and the Chytridiaceae.

Absorption following 'leaking' of the host cell is, for bacterial parasites, apparently the common method in both plant and

animal hosts. It is the method described by Bachmann (1913) for *Bacillus amylovorus*, by Riker (1923) for the crown gall organism in tomato stems, and by Wolf (1924) for the bacterial pustule of soy bean.

The root-tubercle organism has a different life history. Its morphology and physiology have presented difficult problems to both pathologist and physiologist since Eriksson observed the tubercles in the Leguminosae. The early history of the work is adequately outlined by Dawson (1899). She states that Beijerinck (1888) finally isolated the organism and named it *Bacillus radicicola*, and that he concluded that the much debated 'filaments' were composed of bacteria embedded in mucilaginous cell walls, by means of which they are protected from the action of the cell-plasm. Peirce (1902) in his work upon the root-tubercles of Bur Clover gives a different interpretation of the contact conditions. Peirce used a combination of the Gram and the triple stains, whereby the positive-staining bacteria appear purple in the host cells against the orange of the host cytoplasm. In the later stages he shows the two colors fused to a dull gray with no demarcation between the two protoplasms. He states of an infected cell that the cytoplasm is crowded with bacteria. Thus Peirce describes an intimate host-parasite contact even at the stages when others have said the bacteria are discharged from the jelly into the host cell cavity. The existence of the condition described by Peirce calls for further study, but if confirmed the condition would seem to imply a relation between host and parasite protoplasm that prevents their forming surface films when in contact. Buchanan (1909) demonstrated the zoogloeal matrix to be of carbohydrate nature, arising from the diffluent cell walls of the bacteria, and he found a certain degree of racial differentiation in the varying ability of the legume organisms to form this gum.

Certain highly specialized parasitic slime molds and a series of parasitic Chytridiaceae include perhaps the most carefully studied cases of intracellular parasites. These slime molds are apparently for a time parasitic in the host cells without killing them. Nawaschin (1899) describes the symbiotic relationship between *Plasmodiophora Brassicae* and the cells of the host as long continued. The fungus lives, he says, only in the vacuoles and seemingly does not disturb the normal life functions of these

cells until, just before spore formation, the host nuclei and cytoplasm are crowded to the walls by the increase of amoebae. Ward (1901) refers to this fungus as a contrast to the saprophytic *Pythium*. The presence of the entire protoplasmic body of one organism in the cell of another, 'each living its own life for the time being, but the protoplasm of the host cell showing clearly by its abnormal behavior that the presence of foreign protoplasm is affecting its physiology,' is a case, he says, 'of tolerance on the part of the host which adapts itself to the altered circumstances by marked adaptations.' Osborn (1911) reports for *Spongospora subterranea* the same absence of disturbing effects upon the host during the amoeboid life of the fungus.

Lutnam (1913) describes an intimate relation between the plasmodium of *Plasmodiophora* in its early stages and the host protoplasm: 'The plasmodium of the fungus is clear and almost transparent at first, the outlines being difficult to distinguish as they blend with the protoplasm of the host.' The older plasmodium, he says, may be recognized by its abundant oil globules and practically fills the cell lumen, pushing the host protoplasm to the wall. The infected cells are stimulated to hypertrophy and hyperplasy, but only upon approach to spore formation is there degeneration of the host nucleus. Kunkel has worked with both *Spongospora subterranea* (1915) and *Plasmodiophora Brassicae* (1918). He agrees with the reports of earlier workers in the matter of mutualism, but he describes closer contact conditions between fungus and host protoplasm, an actual mingling, as it seems to him, of the two. He shows drawings of *Spongospora subterranea* invading healthy tissue, pushing down between the cells, and finally infecting them by sending small protoplasmic strands through the softened cellulose walls. He did not determine the method by which the plasmodium penetrates the host protoplasm, but he shows stages where the plasmodium has not gone far into the protoplasm; where it has just reached the host nucleus; and where it completely surrounds the latter, apparently directly in contact with the nuclear membrane. Kunkel opposes the earlier conceptions of Nawaschin: 'The plasmodium in the host cell does not appear to be in a vacuole or to be separated from the host protoplasm by a membrane of any kind. The one seems to be somewhat miscible in the other.' He states that the invaded cells are stimulated by the plasmodia to increased

growth and division, but that during all this activity the host nucleus shows little distortion and can sometimes be found intact within the spore balls, a further proof of the specialized parasitism of *Spongospora subterranea*. For *Plasmodiophora Brassicae*, Kunkel traces, in the cortex and the cambium of the cabbage, successive stages in the penetration of the protoplast by the plasmodium. In both these fungi, Kunkel states, the symbiotic relation between host and parasite is so balanced that most of the injury is due to secondary causes. Clearly, these three intracellular organisms, *Pseudomonas*, *Spongospora*, and *Plasmodiophora*, show a high state of mutualism in their relations to their hosts, and they all agree in showing close contact relations between the host cells and the invading protoplasts.

Certain Chytridiaceae show perhaps the most extended host-parasite contact relations reached by intracellular parasites. Such a case is that of *Physoderma Zeae* as described by Tisdale (1919). The delicate outgrowths from the zoospores penetrate the epidermal cell walls of the corn, and thence fine fibers invade a number of the surrounding parenchyma cells, forming within these cells 'enlarged cell groups.' In Tisdale's figures these 'Sammelzellen' seem embedded in the host protoplasm, and according to his report, they have no defined boundary. The host cells appear at this time to be merely slightly enlarged, but by the time the invading bodies have developed into sporangia, the host cells are dead and their protoplasm is almost completely destroyed. As in the case of the Myxomycetes, the mutualism breaks down upon sporulation.

The climax of development of these intracellular growths attained by the parasitic Chytridiaceae is found in *Urophlyctis Alfalfae* as described by Jones and Drechsler (1920). Their figures show the extreme of intracellular parasitic growth processes. From the invaded epidermal cell the processes of the fungous protoplast spread through the sub-adjacent cells of the parenchyma. The resting spore also develops a zone of repeatedly branched projections. Their figures show the host protoplasm close against these branching processes of the fungus. It is by no means a clear case of invagination in the drawings, and Jones and Drechsler do not state their opinion upon this point, but they state the lobes function in the assimilation of food material for the spore. On this ground the increased contact surface

which the processes afford justifies their phrase, 'branched haustorial processes.'

According to the detailed figures of Leitgeb (1881), *Completoria complens* branches within the cells of fern prothalli into a lobed mass much like that of *Physoderma*, and like it, sends processes into neighboring cells. Here, however, the host sets up a definite reaction. A thickening of the host cellulose seemingly delays penetration and later sheaths the base of the process. This seems to be a clear case of invagination. Leitgeb was able to see chloroplasts carried by the streaming protoplasm of the host around the invading body. Whatever the relationship of this little parasite, whose structure is superficially so like that of the Chytridiaceae, its symbiotic adaptations are more like those of the higher fungi, where the haustorium is so general in occurrence.

Fungous parasites producing haustoria

The occurrence of haustoria

As reported in the literature, haustoria occur in certain species at least of all the great groups of hyphal fungi, the Oömycetes, Zygomycetes, Ascomycetes, and Basidiomycetes. The instances which I have found reported in the literature are listed below, arranged according to the classification of Engler and Prantl's Natürlichen Pflanzenfamilien (1897–1900). A comparison of these occurrences affords specific evidence on the question as to whether the existence of haustoria may be considered an index of the grade of specialization in the relation of host and parasite. In the Zygomycetes, their occurrence is limited to one small division. In the three great groups, the Oömycetes, the Ascomycetes, and the Basidiomycetes, there are subdivisions in which their occurrence is practically universal.

Oömycetes:

Pythium palmivorum, E. J. Butler (1910). The only example of haustoria reported for this group.

Albugo candida; haustoria were probably first described in this species; DeBary (1863).

Peronosporaceae; haustoria are reported for practically all genera of this group.

Zygomycetes: In this predominately saprophytic group haustoria are reported only in the cases of *Piptocephalis*, *Syncephalis*, and *Chaetocladium*, the forms for which Van Tieghem (1873) coined the term facultative

parasite. The haustorium of *Chaetocladium*, as described by Burgeff (1920), is more exactly an intermediate cell, in which cytoplasm and nuclei of host and parasite are intimately mingled.

ASCOMYCETES:

Taphrina maculans, Butler (1918).
Coccomyces (Cylindrosporium Padi), Higgins (1914).
Hysterostomella, Lembosia, Parmularia, Arnaud (1918).
Meliola, a genus possessing highly specialized haustoria, Maire (1908), Arnaud (1918), Doidge (1920).
Irene, Doidge (1920).
Erysiphaceae; haustoria are reported for practically all genera of this group, including the probable first mention of haustoria by Visiani (1851) and the detailed work by G. Smith (1900).
Parodiella, Arnaud (1918).
Dimerosporium, Arnaud (1918).
Asterina, Maire (1908), Arnaud (1918).
Acanthostigma parasiticum, Ducomet (reported by Butler, 1918).
Trichosphaeria parasitica, Hartig (1882).
Trabutia quercina, Arnaud (1910).
Podocapsa, Van Tieghem (Reported by Engler and Prantl, 1897).

FUNGI IMPERFECTI:

Darluca filum, Sappin-Trouffy (1896), Clinton (1904), Adams (1920).
Septogloeum Hartigianum, Hartig (1892).
Cylindrosporium Padi, Higgins (1914).
Tuberculina persicina, Sappin-Trouffy (1896).

MYCELIA-STERILIA:

Cuticularia Ilicis, Ducomet (1907).

BASIDIOMYCETES; HEMIBASIDII: In general haustoria are regarded as typical for the group. Lutman (1910) and Sartoris (1924) claim that the presence or absence of haustoria is a distinguishing characteristic between the two main groups. The Ustilaginaceae, they state, lack haustoria, while the Tilletiaceae have haustoria. They correlate this structural difference with a physiological difference. *Ustilago*, they note, infects growing points where food is easily accessible. *Tilletia* occurs in leaf and stem tissues where food is less concentrated. The following reports indicate that this generalization is not absolute for the Ustilaginaceae. Reports agree upon the presence of haustoria in the Tilletiaceae.

USTILAGINACEAE; Absence of haustoria:

Ustilago Zeae-Mays, Knowles (1889), Lutman (1910).
U. laevis, Lutman (1910).
U. Tritici, Butler (1918).
U. Hordei, U. Heufleri, U. Tritici, U. Zeae, Sartoris (1924).

USTILAGINACEAE; Presence of haustoria:

Ustilago Carbo, U. hypodytes, U. longissima, U. Maydis, Fischer von Waldheim (1869).
Ustilago, many species, Tubeuf (1897).

U. Sorghi (broom-corn), Clinton (1897).
U. Zeae-Mays, Guttenberg (1905).
U. striaeformis, Osner (1916).
U. Avenae, U. Hordei, Butler (1918).
Cintractia Seymouriana, P. Magnus (1867).
Sorosporium Saponariae, Fischer von Waldheim (1867), Tubeuf (1897).

PROTOBASIDII; UREDINALES: The two reports following are the only cases I have found in which haustoria are said to be lacking:
Aecidium Urticae on *Urtica dioica*, Schroeter (1875);
Coleosporium Pini on *Pinus virginiana*, Galloway (1896).

The morphology of haustoria

There are occasional references in the literature which imply that the haustorium is an independent cell. Sappin-Trouffy (1896) in some cases finds a partition at the base of the haustorial stalk in *Puccinia graminis* on oats. G. Smith (1900) states that such septa occasionally present themselves, and he figures two cases for *Erysiphe graminis*. R. E. Smith (1905) figures the haustoria of *Puccinia Asparagi* as stubby branches partitioned off from the hyphae. Guttenberg (1905) states that the branched and bent haustoria of the corn smut are abundantly septate. Colley (1918) is undecided as to whether the haustorium of *Cronartium ribicola* is always cut off from the hypha which forms it. Dodge (1923) writes of the haustoria of *Gymnoconia interstitialis* on the dewberry:

> The haustoria are often provided with short, more or less twisted and intertwined branches; in this case they are composed of several cells, each cell with a single nucleus. Such complex haustoria are more in the nature of intracellular hyphae, and nearly fill the cell attacked.

The more general view of the haustorium as a protrusion from a hyphal cell is held by Allen (1923):

> The empty haustorium mother cell remains plump. . . . This would suggest that when the cell contents entered the host cell to form the haustorium, at least a thin membrane was left behind lining the cell wall of the mother cell, and that this membrane is continuous through the membrane of the haustorium inside the host cell.

In a few cases haustoria with broad necks have been described. The absence of constriction is like that found in some penetrating hyphae of facultative parasites, such as *Botrytis* and *Rhizopus*. Sappin-Trouffy (1896) figures a broad neck for the haustorium of *Uromyces Ficariae* on *Ficaria ranunculoides*. The haustoria described by R. E. Smith (1905) for *Puccinia Asparagi* seem merely stubby hyphal branches. There is no evidence of

constriction either in the early protuberance or the later short blunt lobe. Blackman (1904) figures a broad neck for the haustorium of *Phragmidium violaceum* on *Rubus fruticosus*.

The slender neck is, however, most typical for haustoria. Sappin-Trouffy (1896), with reference to *Uromyces Erythronii*, speaks of the narrow hollow pedicel of the haustorium, which gives some communication with the cavity of the hypha. Exceptional length in slender stalks may be noted in such forms as *Hemileia vastatrix* (Ward, 1882), *Erysiphe Cichoracearum* (G. Smith, 1900) and *Meliola amphitricha* (Doidge, 1920).

The great variety of form found in the absorption sac is evidently an individual working out by the parasites of a differentiation to effect increased surface of contact.

These forms fall readily into the four classes of haustoria, which Arnaud (1918) distinguishes in his studies on the Asterineae. They are as follows: (1) The simple haustorium, spherical, pyriform, reniform, straight or bent. (2) The spiral haustorium, derived from class 1 by a lengthening and spiral rolling of the type. (3) The digitate haustorium, where diverticula extend more or less at right angles from the central stalk. (4) The coralloid haustorium, formed of short clustered ramifications from a basal stalk.

Among the many species described by Arnaud the following have especially complicated haustoria: *Asterelibertia Conepiae*, *Parodiopsis Ingarum*, *Trabutia quercina*, *Lembosia Melastomatum*, *Patouillardia clavispora*, and *Perisporiopsis splendens*.

The coralloid type, so abundant in the Asterineae, and also, according to Lutman, abundant in the Ustilagineae, is the rare form in the Uredineae. Sappin-Trouffy's descriptions of over thirty rusts do not afford an illustration. The only good example which I find reported is that of *Botryorrhiza Hippocrateae* (Olive, 1918).

Variations of form in haustoria as influenced by host conditions are reported in many instances in the literature. Nuclear contact as a form stimulus is indicated by Vuillemin's description of a *Puccinia* upon *Thesium* (1894). The haustoria when remote from the host nucleus are simple vesicles; when in contact with the nucleus they branch from a broad basal sac into a tuft of short lobes. Sappin-Trouffy (1896) records variations for rusts in different tissues. *Puccinia graminis* shows simple claviform

haustoria in the sclerenchyma of oats, and twisted, irregularly branched forms in its parenchyma; while *Puccinia graminis* in the barberry shows both types of haustoria in the parenchyma. A similar variation of form in one mycelium infecting different tissues is given by Evans (1907). He finds that the haustoria of *Puccinia glumarum* in the cells surrounding the vascular bundles of the host are much branched, while those in the chlorenchyma are small club-shaped bodies. An abnormal haustorial form due apparently to age of the fungus is described by Tischler (1911) in infected rhizomes of *Euphorbia Cyparissias*, from which all intercellular mycelium had died out and disappeared. These haustorial remains resemble a knot of tangled hyphae, showing clearly septation and a binucleate condition of the cells. Tischler fitly calls this an intracellular haustorial pseudoparenchyma. Arnaud (1918) also notes a variation in different host tissues. He reports that *Lembosia Melastomatum* forms digitate haustoria in the palisade cells of its host, but forms simple elongate sacs in the sclerenchyma fibres. Arnaud reports that the variety of form found in haustoria of the Asterineae is much greater in dicotyledonous than in monocotyledonous hosts. Dodge (1922) comments upon the different forms of haustoria of *Gymnosporangium clavipes* in its two hosts. He finds in the tissues of *Crataegus* coils of irregular hypha-like haustoria; in *Juniperus*, trim binucleated haustoria.

The probable importance of the nucleus of the haustorium is commented upon by Harper (1896) in a study of *Sphaerotheca Castagnei*. According to reports in the literature, the number of nuclei in the haustorium varies greatly. Sappin-Trouffy (1896) figures a digitately branched haustorium for *Uromyces Ficariae*, with two nuclei in the broad stalk and other nuclei in the lobes. Holden and Harper (1903) show, in figures of *Coleosporium Sonchi-arvensis*, a binucleate haustorium, and another where one of the conjugate nuclei is still in the extracellular hypha, concluding that without doubt the nuclei are plastic enough to be elongated in order to pass through the haustorial neck. Gibson (1904) reports that haustoria of *Uredo Chrysanthemi* have often as many as five nuclei. Evans (1907) reports as many as five nuclei in the haustoria of *Puccinia glumarum*. This is interesting in connection with his statement that the hyphae of this rust are crammed with nuclei. The two nuclei which Lutman

TABLE I

Number of nuclei in the haustorium in the gametophytic and sporophytic generations

DATE	AUTHORITY	SPECIES	HOST	GAMETOPHYTE	SPOROPHYTE
1896	Sappin-Trouffy	Coleosporium Senecionis	Senecio vulgaris		2
		Gymnosporangium Sabinae	Juniperus Sabina		1
		G. Sabinae	Pirus communis	1	
		Melampsora betulina	[Betula]		2
		Puccinia fusca	Anemone nemorosa	1	
		P. graminis	Avena		1 or 2
		P. graminis	Berberis	1	
		Peridermium Pini	Pinus sylvestrus	1	
		Phragmidium subcorticium	Rosa		2
		Uromyces Betae	Beta		2
		Uromyces Erythronii	Frittilaria Meleagris	generally 1	
1900	W. Magnus	Puccinia leucospermum	Anemone nemorosa	2	
1903	Holden and Harper	Coleosporium Sonchi-arvensi	Solidago and Aster		2
1904	Ward	Uredo dispersa	Brome grasses		usually 1
1904	Blackman	Phragmidium violaceum	Rubus fruticosus		2
1907	Evans	Puccinia glumarum			
		P. graminis			2 or more often
					5
1911	Tischler	Uromyces Pisi	Euphorbia Cyparissias	1	
1916	Clinton and McCormick	Cronartium ribicola	Pinus Strobus	1 or 2	
1917	Kursanov	Melampsora Pyrolae	Pyrola rotundifolia		2
1918	Colley	Cronartium ribicola	Pinus Strobus	1	
		C. ribicola	Ribes		2
1922	Dodge	Gymnosporangium clavipes	Juniperus virginiana		2
1923	R. Allen	Puccinia graminis Tritici	Triticum		2
1923	Thurston	Gymnosporangium bermudianum	Juniperus barbadensis	1*	1*
			J. virginiana		
			J. bermudiana		

* 'In *Gymnosporangium bermudianum* the two kinds of mycelium exist side by side in a very close relationship within a single gall and each produces its spore form apparently unhampered in any way by the presence of the other . . . . The haustoria of *G. bermudianum* . . . both from gametophytic and aporophytic mycelium contain regularly one nucleus.'

(1910) shows as characteristic of the huge haustorium of *Entyloma Nympheae*, and of the small knobbed haustorium of *Urocystis Anemones*, lie in the broad stalk from which the many branches rise. Lutman suggests that this is probably the most favorable position for the performance of functions in relation to the whole haustorium. Istvánffi and Palinkás (1913) find a single nucleus typical for the haustorium of *Plasmopara viticola*, but in the larger haustoria they find often two, three, or even four nuclei. Only one of these, they say, migrates into the haustorium, the rest being formed by division in the haustorium. The huge coralloid haustorium of *Botryorrhiza Hippocrateae*, as described by Olive (1918), is multinucleate. Allen (1923) calls attention to elongated nuclei in the hyphal runners of *Puccinia graminis Tritici*. She thinks that those which Evans interpreted, in the runners of cereal rusts, as cases of amitosis must also have represented a normal condition. I have often found these elongated nuclei in the hyphae of the corn rust. Their form makes the entrance of nuclei through the narrow necks of the corn haustoria seem very possible.

Table 1 gives the number of nuclei as reported in the literature for haustoria arising from presumably gametophytic and sporophytic mycelia.

Most authors agree that there is a wall around the haustorium, although there are differences of view as to the relation of age to the thickness of the wall. It is possible that the haustorium sheath is not always distinguished from the wall. The following authors give varying reports as to the thickness of the wall. Sappin-Trouffy (1896) describes a thin membrane bounding the vacuolate granular protoplasm of the branched haustoria of *Uromyces Erythronii*, and a thick membrane around the haustoria of *Coleosporium Senecionis*. In the case of *Uromyces Betae*, whose haustoria vary through simple, branched, and corkscrew forms, he notes an increasing thickness of wall in direct ratio to complexity of form, until the membrane of the corkscrew forms is thicker than that of the hyphae. G. Smith (1900) distinguishes a haustorium wall as well as a sheath of altered host cellulose. Maire (1908) distinguishes a membrane for the haustoria of *Meliola* and *Asterina*. For *Meliola* he describes a brown membrane with a distinct lumen for the stalk, and a thin hyaline membrane for the ampulla. For *Asterina* he describes

a hyaline, but often thick, layered membrane around the hyphal branches of the knotted haustorium. Istvánffi and Palinkás (1913) state that the membrane of the haustorium of *Plasmopora viticola* is somewhat thicker than that of the hyphae. Doidge (1923) figures for *Irene matulensis* the same slender-stalked type of haustorium that Maire has shown for *Meliola* but states that its brown walls are thinner than those of the mycelium. Allen (1923) mentions for the haustoria of *Puccinia graminis Tritici* a delicate limiting membrane.

The following authors relate the thickening wall to aging of the haustorium. Ward (1882), describing the haustoria of *Hemileia vastatrix*, states that in older specimens a distinct wall is evident. Evans (1907) notes for *Puccinia glumarum* that as the haustoria become older their walls become thick and stain deeply. Butler (1907) states for *Sclerospora graminicola* that the young haustorium has a thin wall before the deposit of a cellulose cap. Colley (1918) states that the wall of the haustorium of *Cronartium ribicola* is at first quite thin, but that it thickens as the sheath develops.

Relations between haustorium and host cell

Invagination. I have found no definite claim in any case that the haustorium penetrates the primordial utricle and lies in the central vacuole of the host cell. The following workers make no mention of the relation, and show host cytoplasm in their figures indefinitely or not at all:

Anderson, *Aecidium elatinum* (1897); Evans, Cereal rusts (1907); Stämfli, Rust galls (1909); Pritchard, *Puccinia graminis* (1911); Reed and Crabill, *Gymnosporangium Juniperi-virginianae* (1915); Osner, *Ustilago striaeformis* (1916); Arnaud, Perisporiaceae (1918); Adams, Peridermiums (1919); Weston, *Sclerospora philippinensis* (1920); Whitehead, *Urocystis Cepulae* (1921); Moss, Pucciniastreae (1926).

The following workers describe or figure invagination of the host cytoplasm by the haustorium:

Leitgeb, *Completoria complens* (1881); DeBary, The adapted parasite (1884, 1887); Groom, Mycorrhiza on *Thismia Aseroe* (1895); G. Smith, Erysipheae (1900); Ward, *Uredo dispersa* (1904); Tischler, *Uromyces Pisi* (1911); Klebahn, Haustorial fungi (1912); Istvánffi and Palinkás, *Plasmopora viticola* (1913);

Colley, *Cronartium ribicola* (1918); Dodge, *Gymnosporangium fraternum* (1918); Olive, *Botryorrhiza Hippocrateae* (1918); Allen, *Puccinia graminis Tritici* (1923); Thurston, *Gymnosporangium bermudianum* (1923).

Contact between haustorium or other intracellular cells of the parasite and the host nucleus. The question of contact between haustorium and host nucleus has received much attention. The following lists summarize the claims upon the subject in the literature.

No definite contact between haustorium and host nucleus is reported by Werner Magnus, Mycorrhiza in *Neottia Nidus-avis* (1900); G. Smith, Erysipheae (1900); Holden and Harper, *Coleosporium Sonchi-arvensis* (1903); Moss, Certain species of Pucciniastreae (1926).

Occasional contacts, without evidence of any constancy, are reported by Vuillemin, *Puccinia Thesii* (1894); Ward, *Uredo dispersa* (1904); Blackman, *Phragmidium violaceum* (1904); Guttenberg, *Uromyces Alchemillae* (1905); Colley, *Cronartium ribicola* (1918); Dodge and Adams, *Peridermium cerebrum* (1918); Adams, *Peridermium Comptoniae* (1919).

Definite contact relations between host nucleus and haustorium or intracellular cells of parasite are reported by Rosen, *Puccinia asarina* (1893); Groom, Mycorrhiza on *Thismia Aseroe* (1895); Harper, *Sphaerotheca Castagnei* (1896); Sappin-Trouffy, Uredineae (1896); Magnus, *Puccinia leucospermum*, *P. Xanthii*, *Roestelia cancellata* (1900); Peirce, *Pseudomonas radicicola* (1902); Němec, Mycorrhiza in *Calypogeia* (1904); Whetzel, *Peronospora Schleideniana* (1904); Guttenberg, *Puccinia Adoxae*, *Ustilago Maydis* (1905); Evans, *Puccinia Sorghi* (1907); Lutman, Ustilagineae (1910); Zach, *Puccinia glumarum*, *P. graminis* (1910); Arzberger, Mycorrhiza in *Myrica cerifera*, *Eleagnus argentea*, and *Ceanothus americanus* (1910); Tischler, *Uromyces Pisi* (1911); Clinton and McCormick, *Cronartium ribicola* (1916); Allen, *Puccinia graminis Tritici* on Khapli emmer (1926); Moss, *Thecopsora Vacciniorum* on *Vaccinium canadense* and *V. pennsylvanicum*, and other species of the Pucciniastreae (1926).

Sheathing of the haustoria or other intracellular cells of the parasite. The presence of sheaths which are apparently deposits of cellulose from the host cell has been described by De Bary (1863) for the penetrating germ tubes of *Peronospora Radii*,

and by Leitgeb (1881) for the penetrating filaments of *Completoria complens* in fern prothalli. Brefeld and Istvánffi (1895) figure smut-infected oats, and show holes bored in the cuticle, below which the ensheathed germ-tubes may be followed. Lutman (1910) also describes this germ-tube sheath for *Ustilago laevis*.

Sheathed intracellular hyphae are characteristic of many Ustilagineae. Sheaths enclosing the invading hyphae in certain Ustilagineae were reported by Fischer von Waldheim (1869). His descriptions include *Ustilago Maydis*, *Ustilago Carbo*, *Sorosporium Saponariae* and *Urocystis occulta*. The mycelial strands, he says, are completely encased by a thick cellulose sheath. Reinhold Wolff (1873) describes the same phenomenon for *Urocystis occulta* in rye. Lang (1899), reporting upon the endophytic fungus in the gametophyte of *Lycopodium clavatum*, states that the hypha in passing through the basal wall of the rhizoid is surrounded by a short sheath derived from the perforated cell wall. Guttenberg (1905) has not only verified sheathing for the corn smut, but sees in it a protective reaction of the host plasm controlled by the nucleus.

DeBary (1863) figures a sheath about the haustorium of *Cystopus candidus* in cortical parenchyma of *Tragopogon*, and about the haustorium of *Peronospora Umbelliferarum* in a leaf of *Aegopodium Podagraria*. After describing the haustoria of *Aecidium elatinum* (1867) he adds that many of them fail to penetrate the walls of the host cells and are covered by a thickened, shining sheath. DeBary (1884) explains the cellulose sheath as due to the continued action of a pellicle of the living host cell, which forms a close sheath around the intruder.

Mangin (1895) reports that the haustorial sheaths in the Peronosporeae consist of a cellulose more resistant than ordinary, by reason of the association of a second substance, callose, with the cellulose. Ordinarily, he says, the haustoria have a double envelope, and between these two one often sees irregular and voluminous masses of callose, which at times cause the rupture of the external membrane. At other times he finds that the outer membrane consists only of callose and forms a complete sheath around the haustorium, sometimes so massive as to crowd the host protoplasm to the wall.

By far the most detailed study of sheath formation has been

made by G. Smith (1900) working on the haustoria of the Erysipheae. Smith reports that the first stage of penetration by a haustorium is indicated on the outer surface of the leaf by the appearance of a clear shining area around the point of penetration. At the same stage the inner surface of the cell wall immediately under the point where the hypha comes in contact with the epidermis stains deeply. The wall thickens over an area coinciding roughly with the clear space noted. Smith holds that the density of the cellulose indicates actual growth in thickness, rather than swelling, such as Ward describes for *Botrytis*. This thickening of the wall for some time keeps in advance of the growing tube of penetration, forming a knob-shaped papilla in the cell, but gradually, he believes, the haustorium digests this. Finally the mature haustorium lies in a cavity filled with products of the dissolved cellulose sheath and bounded by the invaginated plasma membrane. Around its slender stalk a collar of cellulose remains, which seems continuous with the cell wall.

A very remarkable cellulose formation is described by Němec (1904) for *Calypogeia Trichomanis* stimulated by mycorrhizal fungi. The fungus forms a pseudoparenchymatous tissue of intracellular hyphae, from which slender haustoria penetrate in such numbers into adjacent cells that a surface view of a wall so entered looks like a sieve. In many cells the haustoria never perforate the cellulose projections which sheath them. Often a whole wall may thicken to inclose a series of haustoria.

Ducomet (1907) describes a heavy sheath with a roughened granular surface around the haustoria of the subcuticular fungus *Cuticularia Ilicis*.

In the case of the East Indian Maize mildew, *Sclerospora graminicola*, Butler (1907) finds both the hyphae and the haustoria sheathed. The invasion hyphae are similar to those which Wolff described for the smuts. They run intracellularly but are so heavily walled that the lumen is almost occluded. Butler figures peg-like processes projecting from these hyphae into the cells, but apparently arrested by a cellulose cap. He distinguishes, as does Mangin, two layers in the sheath. He does not, however, identify callose and cellulose, but specifies that the inner layer belongs to the fungous hypha and the outer layer is deposited by the host cells 'in the effort to stop the fungal growth.'

Stämfli (1909) compares a rust-caused gall on *Rubus* with that reported by Guttenberg as due to the infection of the Alpine rose by *Exoascus Rhododendri*. In the latter the mycelium is limited to the first and second subepidermal layers, and the haustoria are sheathed with cellulose. The *Rubus* gall is completely penetrated with mycelium and the haustoria are without 'zellulose Kappe.'

Tischler (1911) describes cellulose sheaths around the older haustoria of *Uromyces Pisi* in the rhizomes of *Euphorbia cyparissias*.

Němec (1911) states that in tissue infected by *Uromyces Betae* the host plant many times covers the haustorium with a sheath while at other times the haustoria remain naked.

Higgins (1914) reports that the haustoria of *Cylindrosporium Padi* are enclosed by a sheath.

Colley (1918) regards the cellulose sheath of the haustorium of *Cronartium ribicola* on *Pinus Strobus* as an accompaniment of maturity or old age. The sheath forms a long cup-like holder at the base of the haustorium and a thick cap over the tip, while over the middle region it is very thin. Colley explains this peculiarity as the result of response by the host to two chief points of irritation: the point in the cell wall through which the haustorium entered, and the point of contact of the advancing haustorial tip with the host cytoplasm. Colley states that the haustoria of the mycelium in *Ribes* are not sheathed, that they are smaller and proportionately less frequent, on a less crowded mycelium.

Arnaud (1918) does not mention sheath formation in his studies on the Asterineae, but he shows such a structure in a few figures. In figures of the haustoria of *Meliola Andirae Puttemansii* and of *M. nidulans*, the slender penetrating neck is shown piercing the cuticle and the characteristic ampulla is encased in the indented epidermal wall. In the figure of the haustoria of *M. polytricha* forma *Anacardiaceae*, the filament has passed between epidermal cells to the palisade layer and the ampulla is encased in the wall of a palisade cell.

Weston (1920) reports sheathed haustoria for the two species of downy mildew which he has investigated in the Philippines, *Sclerospora philippinensis* and *S. spontanea*.

Thurston (1923) reports a noticeable sheath about the haustorium of *Gymnosporangium bermudianum*.

Allen (1923) describes a glistening, transparent sheath around dead haustoria of *Puccinia graminis Tritici*, form 3, on Mindum wheat. She concludes:

> It seems probable that the sheath originates from host cytoplasm, although the possibility is not excluded that the haustorium itself contributes to it by secretions of some sort, or that host, or fungus, or both, secrete more or less of cell wall substances around the haustorium.

Moss (1926), reporting upon fourteen species of Pucciniastreae, finds in most of the species disorganized haustoria enclosed in sheaths, which in staining properties resemble the cellulose of the host cell-wall. He thinks the sheath is laid down by the host cytoplasm in response to the stimulatory action of the haustorium. In *Milesina marginalis* and *M. polypodophila* he finds frequently a basal collar, which he considers an early stage in the development of the sheath.

Metabolic relations between host cells and haustoria. It is generally assumed that the rust works harm to its host by its increasing demands upon the food and water supply. Results which in other cases are often ascribed to toxic products are, with the rusts, commonly assumed to be merely culminating effects of inanition. Ward (1890) in his Croonian lecture points to the Uredineae as proof of the statement, 'In all cases where haustoria are developed, the mycelium enters into a peculiar symbiotic connection with the cells, and for some time merely taxes them as it were, rather than injures them directly.' Fromme (1913) suggests that the rust fungus 'is dependent on the transition products in photosynthesis.' Mains (1917) concludes from tests with *Puccinia coronata* and *P. Sorghi* that 'the rust is dependent upon the food supply of the host, and does not live upon its protoplasm.'

Reports of food disappearance in the region of haustoria as given in the literature support these statements. Butler (1907) reports in the case of *Sclerospora graminicola* that starch is absent in cells with haustoria even before the assimilatory activity at the time of spore formation, when the cells of the host collapse and die. Reed and Crabill (1915) ascribe to the presence of haustoria the disappearance of starch in the cells of cedar-apple tissue. The comparative studies of Mains (1917) upon stages in the development of *Puccinia Sorghi* demonstrate inanition as the outstanding effect of the rust upon its host. He gives specific

data upon food disappearance from the host cells over a period extending from the third day after inoculation, when the mycelium was well developed, through the sixth day, when the first pustules appeared. The only abnormal effect which he observes in the host cell is a decrease of the storage starch in the parenchyma sheaths of the vascular bundles. During the drain incident to spore formation he finds the sheath cells in the vicinity of the pustules entirely devoid of starch. 'Yet even at this time,' he observes, 'the cells of the host do not show an injury such as one would expect if the protoplasm itself was attacked vigorously by the fungus.' This inanition effect on the corn is the more striking because, as Mains reports, the sheath cells at the time of their depletion are not invaded by haustoria. He concludes: 'It would appear that this loss of starch is due, not to a withdrawal of starch from the parenchyma sheath by the fungus itself, but to the utilization by the fungus of the material formed in the neighboring region before it reaches the parenchyma sheaths.' Mains notes also a disturbance in the normal water currents. He refers to the statement of Sachs that loss of water from dead tissue is much greater than from living. He thinks that such evaporation from dead areas developed around green infection centers causes a drain of water from these centers, which effects their ultimate death. This matter of evaporation from dead tissues may have bearings upon transpiration data.

It is difficult to get evidence upon the presence of soluble carbohydrates. Tischler (1911) infers from their high osmotic pressure, determined by plasmolysis, the presence of high sugar content in the vacuoles of infected cells of *Euphorbia Cyparissias*. Long (1919), by applying the Fehling test to extractions of digested leaf contents, has made a series of extensive comparisons of photosynthetic activity in plants under various conditions. Among these she made tests upon seedling oats and wheat infected with *Puccinia coronata* and *P. graminis*, respectively, and upon plants of *Arisaema triphyllum* naturally infected with *Uromyces Caladii*. She concludes that the fungus interferes seriously with the accumulation of carbohydrates by the host, and that sporulation reduces by more than 50 per cent the ability of the host tissues to make carbohydrates. She refers these effects to a possible interference with the transpiration of the host and to the drain of food by the fungus.

On the other hand, an accumulation of food in infected tissue is a well known phenomenon, and may be considered a stimulation effect by haustoria. Barclay (1886) has described swellings occurring upon the Himalayan *Urtica parviflora* infected by *Aecidium Urticae*. These swellings contain an abundance of nutritive material, which the hill natives eat with relish. Under microscopic observation Barclay finds granules both in the hypertrophied cortical parenchyma cells and in the central pith cells. He gives their blue reaction to Schultze's fluid as proof of their starchy nature although they show neither striae nor hila. He states also that they are readily soluble in water, for he misses them in water-mounted sections, but finds them abundantly in glycerine mounts. Barclay concludes that this fungus requires considerable aid from its host for its complete development, as the aecidia are developed only after the reserve food has been amassed. Barclay proved the heteroecism of this rust by infecting nettles with teleutospores from *Carex setigera*, but here again he finds evidence of the unusually heavy demands of the parasite, since seedling nettles were not successfully infected. They were unable, he states, to nourish a parasite that demanded so much. There was no accumulation in the *Carex* generation of the granules mentioned above. Halsted (1898) demonstrated starch in *Podophyllum* leaves infected with *Puccinia Podophylli* by soaking the tissue in iodine. He found the infection areas in the leaf definitely outlined by small veins, and starch was found only within these areas. Guttenberg (1905) gives several examples of storage. The abnormal pistillate clusters of *Alnus incana* infected by *Exoascus amentorum* contain a parenchyma rich in starch, which Guttenberg interprets as a nourishing tissue for the fungus. In *Capsella Bursa-pastoris* he finds evidence of sugar accumulation in the appearance of anthocyan, which precedes the formation of conidiophores. He states as further evidence of the development of a storage tissue that it is into these cells the fungus sends the characteristic small knob-like haustoria. Guttenberg finds still a third type of storage in *Rhododendron* infected by *Exobasidium Rhododendri*. The galls, he states, are water reservoirs. It has been already noted that Tischler (1911) infers high sugar content in cells of *Euphorbia Cyparissias* infected with *Uromyces Pisi*, because of the high osmotic pressure. The result is a thick leaf of xerophytic type.

The chloroplasts are pale and reduced in number. To this diversion of food into leaves which have lessened photosynthetic activity but increased activity in growth, Tischler lays the death or abortion of the vegetative points, which are not in themselves infected. Reed and Crabill (1905) report of the cedar-apples upon *Juniperus virginiana* that during summer, autumn, and early winter the fungus in the gall appears to stimulate the parenchyma of its host to prodigious activity in the multiplication of cells, and the storage of starch, which is used up during teleutospore production. Colley (1918) reports of *Pinus Strobus* infected by *Cronartium ribicola* that storage starch is usually present in excess, and that it is not completely used up by the fungus, as many grains remain in old dead cells after the cells are completely dried out. Colley suggests that excess starch is due to a lack of balance in physiological processes in the host cells—an unbalancing to which the fungus probably contributes, although other little understood environmental factors are known to lead to the production of excess starch in trees.

The observations quoted concerning abnormal accumulations of food have involved, in many cases, mention of hypertrophy and hyperplasy, which are responsible, even more than is food accumulation, for many characteristic deformations of host plants of the fungi. These structural effects of fungus stimulation upon the host occur with all degrees of complexity. The many slight modifications of host tissue, which have been detected by microscopic observation prove that these stimulations are more general than is at first realized. In the large number of cases in the literature which report hypertrophy and hyperplasy no special reference to the relation of haustoria has been mentioned. Their presence is reported in the gall as in any other infected tissue. The presence or absence of haustoria seems to have no bearing upon the meaning of hypertrophy and hyperplasy.

The physiological reaction of an invaded host cell involves translocation of foods, transpiration, and respiration, as well as photosynthesis. Comparatively little has been done to test the interrelation of these processes in rusted plants. Reed and Crabill (1915) have attempted such a study, using as material apple leaves infected with the aecidium of *Gymnosporangium Juniperi-virginianae*. They find, both by the measure of carbon dioxide consumed and by the carbohydrate cell-content, that

the photosynthesis of diseased leaves is less than normal. They find that transpiration is not only lessened, but is a constant, instead of a fluctuating process. However, these transpiration effects, they state, are evidently due to blocking of substomatal cavities through hypertrophy of the host tissue. They find a temporarily increased rate of respiration and liken the action to a fever which wastes away the tissues of the victim. 'Parasitism,' they conclude, 'may be regarded as a type of injury.' This is borne out by their report of the poor growth and the unthrifty condition of the infected trees.

Increased respiration by rusted plants is reported by Nicolas (1920) from tests with *Puccinia*, *Melampsora*, and *Phragmidium*, as well as with *Urocystis* and *Taphrina*. His explanation agrees with that of Reed and Crabill in emphasizing great activity in the host. The fungus, he says, stimulates to hypertrophy and hyperplasy. The consequent inflow of a great quantity of hydrocarbons, indicated frequently by the abundance of anthocyan, realizes conditions favorable for a great increase of respiration. Increased transpiration at the same time tends to counterbalance the excessive turgescence. He reports the reverse in the case of ectophytes.

Decreased respiration is reported by Bailey and Gurjar (1920) for rusted wheat. Their test plants were cut two weeks before maturity. Both heavily rusted and sound specimens were sealed in glass tubes for respiration tests, and were later examined for moisture content. They conclude: 'If the respiration of these tissues is in any sense a measure of metabolic processes it appears that the metabolism was decidedly depressed in the rust infected plants.' They infer increased transpiration on the part of the rusted plants from the fact that their moisture content was much lower than that of sound plants.

Dodge (1923) finds in a case of *Rubus* infected with orange-rust a structural change in the host which might cause increased respiration and transpiration, although Dodge does not relate it primarily to these processes. He reports that when infection develops during the plastic period of leaf tissues, stomata develop over the infected areas in the upper epidermis, which ordinarily lacks them. This, Dodge believes, is a case of host action in the 'interests' of the fungus. The extra stomata develop only over areas infected by the gametophytic mycelium, and in these areas

they develop before the appearance of the hypophyllous aecidial crust. Apparently then the new structures are not due to abortion of the normal stomata. Dodge concludes: 'The gametophytic mycelium of the rust stimulates the host to provide ready means of access by the sporophytic stage which is to follow later.'

Weiss (1924), after a review of literature dealing with the effect of parasitic infection on the water requirement of plants, concludes: 'Fungous infection may result either in acceleration or diminution of transpiration.' The cases of accelerated water loss are, he thinks, determined by injury to tissues or by the production of toxic excretions; the cases of diminution of transpiration are determined by alterations of the tissues in water absorbing and water excreting parts, by alteration of the osmotic relations of infected cells, and by the reduction of yield of tops and of grain coincident with an increased water requirement. He thinks, however, that increased transpiration is less likely to be the cause of the diminished yield than are destruction of chloroplasts in the infected tissue and the drain on the elaborated food reserves of the host.

These analyses of the causes underlying changes in respiration in infected leaves have not included a consideration of the fact that a part of the increased respiration must be the respiration of the parasitic mycelium. Judging by the gas exchanges which have been measured for various molds, this should be an appreciable amount. Weimer and Harter (1921) compared the relative amounts of carbon dioxide given off from two halves of the same sweet potato, one of which was infected with *Rhizopus Tritici*. It was found that the decayed half gave off a total of from 6.3 to 7.8 times as much carbon dioxide as the healthy half. The mass which respires in the case of the rust-infected tissue is host protoplasm plus parasite protoplasm, as against host protoplasm alone in an uninfected tissue.

The chloroplasts are perhaps the most delicate indicators of the degree of adjustment between host and parasite and the state of metabolism in the host-fungus complex. Destruction of chlorophyll is frequently found even in the case of the haustorial parasites.

A chlorosis which causes white-streaked leaves is a characteristic effect of the Sclerosporas upon maize as described by Butler

(1907), and by Weston (1920). Osner (1916) reports of Timothy smut that the mesophyll cells around the young sorus have lost their chlorophyll contents.

There are also reports of plastid disorganization due to rust infection. In some cases the yellowing may be secondary to hypertrophy. Thus DeBary (1853) reports a disappearance of chlorophyll in tissue infected by *Roestelia cancellata*. Reed and Crabill (1915) report of infection by *Gymnosporangium Juniperi-virginianae* that the palisade cells in the rust lesions contain carotin and erythrophyll but no chlorophyll.

The following seem to be cases where the tissue loses chlorophyll without hypertrophy. Ward (1882) describes as the sign of infection by *Hemileia vastatrix* the appearance of yellow spots, which spread centrifugally and darken to a dead black area. Richards (1896) reports that in cases of infection by *Aecidium houstoniatum* the chlorophyll is mainly disorganized by the fungus. Galloway (1896) reports in the case of infection by *Coleosporium Pini* that the chlorophyll bodies disappear and many large oil bodies are seen in their place. *Puccinia Asparagi*, as compared with other rusts, shows pronounced virulence. R. E. Smith (1905) describes the destruction of the green host tissue by this rust. The chloroplasts, he states, shrivel, collapse, and clump into several masses in the center of the cell. Smith relates this effect to the action of the haustoria, which he figures as unconstricted stubby branches. His report of the reaction suggests the *Botrytis* type. They 'penetrate the cell only slightly, if at all, and immediately the chloroplasts begin to react. . . . Apparently, a soluble substance, poisonous or enzymatic, is excreted to bring about this effect.' As noted above, Tischler (1911) reports of leaves of *Euphorbia Cyparissias* infected with *Uromyces Pisi* that the chloroplasts are pale and reduced in number. Reynolds (1912) reports in the case of infection by *Puccinia Potentillae* that the chlorophyll seems to have largely disappeared from the cells which are within the influence of the rust. Bell (1924) reports that fir needles infected with *Peridermium pycnogrande* turn pale green; those infected with *Peridermium balsameum* are blanched.

Disorganization of chloroplasts is also reported as one of the characteristic effects in rust-infected cells of more or less resistant hosts. It may be in these cases the outward sign of a hypersensitiveness, which, according to Stakman, underlies immunity.

Ward (1904) found distinct differences in the susceptibility of various brome grasses to *Uredo dispersa*. Over against his description of lack of reaction in a cell of a susceptible host, he describes for rust-resistant grass the appearance of pale flecks due to destruction of chlorophyll in the attacked cells, and in extreme cases the blackening and shrivelling of the infected spots, due to actual death of the cells. Marryat (1907), comparing the effects of *Puccinia glumarum* upon the susceptible Michigan Bronze and two more or less resistant wheats, American Club and Einkhorn, reports much the same results. Stakman (1915) writes of the action in cells of oats inoculated with a form of *Puccinia graminis* which does not grow in it normally:

> Within a short time after the hyphae become closely appressed to the host-plant cells, there are usually unmistakable evidences of some deleterious influence upon the host cells. The chloroplasts very often seem to be affected first. They may appear slightly corroded at first and somewhat irregular in outline, they may retain their identity for some time, but more often seem to be clumped together in more or less irregular masses . . . eventually the outlines of the plastids become obliterated almost entirely, leaving only a fairly homogeneous, uniformly staining, non-granular mass with little remaining semblance of structure.

Allen (1923) writes of an infected cell in the resistant Kanred wheat: 'The disordered contents of this cell, the absence of the nucleus, the clumped misshapen plastids, and the rough jagged points on the cell left by the irregularly collapsing wall give ample proof of the attack.' Of plastid reactions to rust in the cells of resistant Khapli emmer she writes (1926): 'The plastids are still discernible and are but little reduced in size, but both in their shape and staining reaction there is evidence of dissolution.'

In contrast to these reports of disorganization in rust infected tissue there are many reports of infection by rusts in susceptible hosts without obvious and immediate pathogenic reactions on the part of the host. Ward (1904) emphasized such a condition for the brome grasses susceptible to *Uredo dispersa:*

> One of the most surprising features of invaded cells is the longevity of their cell contents. Even in preparations of tissue thoroughly infested for some days—the nucleus, chlorophyll corpuscles, and cytoplasm may retain their form, color, and even their normal capacity for staining.

Evans (1907) in his many figures of rust-infected tissue of the cereals shows the chloroplasts in apparently normal position

along the periphery of the cell, no matter what the age of the haustorium. Guttenberg (1911) figures plastids in a rust-infected cell of *Adoxa* lying in the primordial utricle and in the invaginated cytoplasm around the haustorium. Reynolds (1912) states that the chloroplasts of *Viola cucullata*, attacked by *Puccinia Violae*, are little affected. Klebahn (1912) makes the rusts the types for his group of strict parasites which do not cause discolored dead flecks as a sign of infection. Stakman (1915) writes:

> It is probably superfluous to call attention to the fact that when normal infection, such as occurs for example, when oats are inoculated with *Puccinia graminis* from *Dactylis glomerata*, takes place, the host cells remain at least apparently normal for a considerable length of time . . . The hyphae may grow very vigorously, send haustoria into the cell and branch profusely without destroying the chloroplasts or in other ways injuring the cell. Even after pustule formation has begun many of the cells just at the edge of the pustule, where the hyphae are massed in great numbers still retain their chloroplasts . . . .

Doran (1921) states of rust infected *Antirrhinum* that the chloroplasts fade slightly, but the yellow of the leaf fleck is due rather to color in the masses of mycelium. Allen (1923) describes a six-day-old cell of early Baart infected by *Puccinia graminis Tritici* as follows:

> The cell drawn contains two large haustoria, which are intimately associated with the living contents of the host cell, being covered with a rich layer of host cytoplasm. The infected host cell presents a flourishing appearance and may even have more plastids and cytoplasm than an uninfected cell.

When unaltered chloroplasts remain in infected areas in the midst of dying, uninfected tissue, the phenomenon may indicate not merely mutualistic adaptation but host cell stimulation by the fungus. This persistence of chlorophyll in infected regions, causing in Stakman's phrase 'green islands in yellowing tissue,' has been frequently noted, not only among the rusts but in other fungi. I have found the following instances mentioned in the literature.

Uredineae: Figures of *Puccinia coronata* on Avena, and of *P. dispersa* on *Secale*, Eriksson and Henning (1896); *Coleosporium Pini* on *Pinus virginiana*, Galloway (1896); *Cronartium asclepiadeum* on *Vincetoxicum*, and *Gymnosporangium clavariaeforme* on quince, Tubeuf (1897); figure of *Puccinia rubigo-vera Secalis* on rye seedling, Carleton (1899); *Uredo dispersa* on brome grasses, Ward (1902); Uredineae, Arthur (1903); *Uromyces Veratri*, on *Veratrum album*, Morgenthaler (1910); *Puccinia graminis Secalis* on

rye, Stakman (1914); *Puccinia Sorghi* on corn, Mains (1917); *Uromyces appendiculatus* on partially resistant bean leaves, Fromme and Wingard (1919); *Puccinia graminis* on wheat, Long (1919); *Puccinia graminis Tritici* on Khapli emmer, a resistant wheat, Allen (1926).

ASCOMYCETES: *Uncinula Aceris* on *Acer plaianoides*, and *Rhytisma punctatum* on *Acer spicatum*, Tubeuf (1897); *Venturia inequalis* on apple, Butler (1918); *Rhytisma punctatum* on Acer *pennsylvanicum* and *A. spicatum*, Overholts (1926).

CHYTRIDIACEAE: *Physoderma Zeae-Maydis* on *Zea Mays*, Tisdale (1919);

FUNGI IMPERFECTI: *Septoria Apii* on celery, Thomas (1921).

Immunity

An early and still prevalent conception of individual as contrasted to racial immunity associates it with the health and general vigor of the host. Raines (1922) has reviewed the history of this theory and set forth evidence for a counter claim, from the literature, and from his own experiments. His review covers field observations and culture experiments dealing with host nutrition, water relations, temperature relations, stimulants and depressants, trauma, age and maturity of host tissue. He cites the following authorities in support of the concept that in the rust diseases of the higher plants there is a tendency for the parasite to exhibit a higher incidence of infection and greater virulence on the host of greater vegetative vigor: 1883, Little; 1889, Bolley; 1902, 1905, Ward; 1903, 1905, Sheldon; 1903, Galloway; 1903, Arthur; 1904, Gibson; 1905, Christman; 1905, Smith; 1906, Butler and Hayman; 1907, Jost; 1911, Freeman and Johnson; 1912, Peacock; 1912, Biffen; 1912, Johnson; 1912, Voelcker; 1913, Spinks; 1913, Fromme; 1913, Zavitz; 1914, Eriksson and Hammarlind; 1914, 1917, 1919, Stakman; 1915, Hecke; 1917, Mains; 1917, Stakman and Piemeisel; 1918, Butler; 1918, Giddings; 1919, Stakman and Levine; 1919, Lauritzen; 1920, Bailey.

The instance which Raines cites from R. E. Smith's report (1905) upon asparagus rust states that the very quick-growing succulent asparagus which grows upon a peat soil in California lacks resistance to injury and succumbs readily to rust. This may, from the standpoint of luxuriant growth, be interpreted as susceptibility in a vigorous host, but in an earlier article Smith (1904) gives the opposite interpretation for asparagus, stating that an abundance of soil moisture during the summer has a marked effect in retarding the development of rust by giving the host greater vigor and resistance.

I can add to the list of Raines the names of the following men who link the health of the host plant with susceptibility: 1912, Klebahn; 1918, Clinton; 1924, Stakman and Aamodt; 1926, Hart.

The findings of Dickson (1923) for *Gibberella Saubinetii* in seedlings of corn and wheat furnish indirect evidence along the same line, for although they report that the seedlings blight most at temperatures which are different from those of optimum growing conditions for corn and wheat, they claim that susceptibility is not due to ill health of the host, but that the temperatures unfavorable to host growth merely prolong the period during which the seedling sheath is susceptible to penetration by the fungus.

The establishment of the fact that to the obligate parasite host vigor furthers infection brings a new viewpoint in considering the fundamental question as to the nature of immunity. According to Hursh (1924), in his historical review of the subject of rust resistance, the early investigators, Sappin-Trouffy, Cobb, Peterman, and Farrer, saw in protective leaf surfaces, resistant tissues, stomatal characters, and leaf positions a basis for resistance. Eriksson and Henning (1896), Ward (1902), and Biffen (1907) threw the emphasis upon physiological characters as the basis for resistance.

The investigations of Allen (1923) upon wheat rust give serious attention again to the possibility of morphological causes of resistance. She thinks that for Kanred, the resistant wheat studied, 'true immunity' is a physiological character; but she finds two morphological characters which favor resistance—stomata which shut out the majority of the fungi, and heavy contact walls adjoining pathological cells. Hursh (1924), in studies on the resistance of wheat to *Puccinia graminis Tritici*, has set forth data concerning morphological peculiarities of the host in the most detailed study along this line since that of Ward. He concludes: 'Resistance to stem rust must be considered as being due fundamentally to a complex physiological relationship.'

The problem of the nature of the physiological resistance of a host to rust was early approached from the cytological side. Klebahn (1896) made from *Puccinia Digraphidis* Soppitt two biological species, *Puccinia Convallariae-Digraphidis* and *Puccinia Smilacearum-Digraphidis*, on the ground that the first found a susceptible host in *Convallaria* and the second in *Polygonatum multiflorum*. *Puccinia Convallariae-Digraphidis*, in

leaves of the wrong host, *Polygonatum multiflorum*, caused red-brown flecks. Microscopic examination proved that in every such spot sporidia had bored through the walls of epidermal cells, and the germ tube had swollen into an intracellular sac, but, Klebahn states, the fungus was not in a condition in the epidermal cell to develop itself into mycelium in the interior of the plant. Antagonism between the fungus and the host led here, on one side to a killing of the attacked cell, and on the other to a checking of development of the parasite. The work of Ward (1902, 1905) upon *Uredo dispersa* and *Puccinia glumarum*, confirmed by Gibson (1904) upon chrysanthemum rusts, and by Marryat (1907) upon *Puccinia glumarum*, definitely located the infection reaction for rusts as one between the host protoplast and the penetrating haustorium.

This is confirmed from another group of highly adapted haustorial parasites. Salmon (1905) described an experiment with *Erysiphe graminis* to ascertain at what stage and in what manner the action of the 'wrong' host plant stopped the further growth of germinating conidia. Conidia from wheat were used for inoculations upon barley. There were no true infections, but microscopic examination 24 hours after inoculation showed that germ tubes and appressoria had been produced by nearly all the conidia, and that in the majority of cases a penetrating tube had entered an epidermal cell and had formed an incipient haustorium in the cell. This small rounded body, however, was in striking contrast to the normal lobed haustorium of *Erysiphe graminis*, and by the fifth day most of these rudimentary haustoria were completely disorganized. Salmon (1905) made similar tests with the mildews of brome grass, wheat, barley, and oats, and finds that the parasite is hindered by influences of the 'wrong host' from carrying on its normal functions and thus obtaining the food necessary for the production of mycelium. He concludes that 'susceptibility or immunity, at all events in the case of these host-plants and their "biologic forms" . . . depends on the capacity or incapacity for maintaining certain working relations between the haustorium and the host-cell.'

The segregation of biologic species of *Puccinia graminis Tritici* has offered most favorable material for cytological studies of resistance. Stakman (1914) figured results of the struggle of *P. graminis Tritici* in the resistant tissue of Khapli wheat.

Allen (1923) compared reactions of *P. graminis Tritici* in susceptible Baart, and resistant Kanred wheat. Her cytological studies confirm observations of earlier workers, in that she indicates the host cell as the place where resistance is expressed, and the attempted penetration of a haustorium as the act by which resistance is stimulated. Her observations are further elaborated in a series of comparative studies upon several forms of *Puccinia graminis Tritici* in resistant wheat (Allen, 1923, 1926). Hart (1926) finds physiologic specialization in the case of *Melampsora Lini*. She reports that Argentine flax apparently possesses 'a true physiologic resistance. . . . Argentine is hypersensitive to the pathogene, which kills some of the host cells quickly and is then unable to develop further.'

Such observations and descriptions lead of course to the question of the nature of the resistance thus located. The differential mineral culture experiments of Ward (1901), although somewhat indeterminate in result, indicate that resistance is not a matter of nutrition, and after his study of the rusted brome tissues (1905) he concludes in terms of current pathological theory that

> Infection and resistance to infection depend on the power of the fungus-protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins, and reciprocally, on that of the protoplasm of the cells of the host to form anti-bodies which destroy such enzymes or toxins, or to excrete chemotactic substances which repel or attract the fungus-protoplasm.

Gibson (1904) gives Ward's idea in the statement:

> The facts seem to suggest that the death of the entering hyphae is not due so much to starvation as to some poisonous substance emitted by the cells, for on examining an epidermis freshly stripped from a leaf inoculated three days before, the hyphae outside are seen to be alive and unshrunk while a great number of those inside are already dead and shrivelled.

Marryat (1907), also from Ward's laboratory, states:

> We are therefore forced to fall back upon the theory that immunity to disease is due in these cases to the production of certain toxins and anti-toxins by host or parasite or both, which are mutually destructive.

Stakman (1915), as noted above, has developed the conception of hypersensitiveness as the basis of immunity. He states the case as follows:

> The essential fact is that the fungus gains entrance in the same manner in susceptible and resistant forms, but acts differently thereafter. In susceptible forms it grows vigorously without seriously affecting the host cells

for some time. In resistant forms, on the other hand, a very rapid action results in the almost immediate death of the host cells. The degree of susceptibility is indicated to a certain extent by the rapidity of this action The more resistant a form, the quicker are a few host cells in the immediate neighborhood of the invading hyphae killed and the sooner does the fungus itself cease activity.

Allen (1923) concludes from her work upon Kanred wheat:

The observations recorded here are in line with the theory that immunity is due to definite antagonistic chemical interactions between host and parasite.

The history of attempts to correlate the immunity of a given host with the physicochemical properties of its cell sap is reviewed by Hursh (1924). Such studies extend from those of Comes (1909) to the experiments of Hursh himself upon determination of the depression of the freezing point, total solids, average molecular weights, hydrogen-ion concentration, and sugar content of wheat varieties. He concludes:

There are differences in the physicochemical properties of the sap of different varieties of wheat. It has been impossible, however, to make definite correlation between these properties and rust resistance.

This is disappointing in view of Stakman's earlier conclusion (1914) that the question as to the immediate instruments of immunity can probably be answered only by means of biochemical investigations. Possibly Hursh did not study the right properties. In the studies of Allen (1926) upon three forms of *Puccinia graminis Tritici* in resistant Khapli emmer, the conclusions, drawn from the cytological data, all point to chemical secretions as the means by which both organisms cause toxic effects, and as the probable cause of the recovery which occurs in cell groups infected by the less resisted forms. For example she writes:

Perhaps, on the other hand, the secretions of the young fungus set up counter-reactions in the host, by means of which later secretion of the same substance is rendered less destructive.

INVESTIGATIONS: THE HAUSTORIA OF THE UREDINEAE

Puccinia Sorghi Schweinitz

External phenomena of infection

For a study of host-parasite relations in the Uredineae, *Puccinia Sorghi* was chosen because of the ease with which the rust can be grown upon *Zea Mays* throughout the year under greenhouse conditions. Golden Bantam corn proved a susceptible

host, but it did not flourish as well as field corn under plant house conditions. Flint corn was therefore used for the following experiments. It was a susceptible host and grew readily in the plant house. No attempt was made to determine physiologic species. Although for specific tests many inoculations were made by spraying, a progressive supply of material was assured merely by setting successive pots of corn seedlings near rusted corn plants. Cultures were under observation in the Columbia University plant house from 1921 to 1923.

Germination studies of corn rust were made by applying a spore suspension to a leaf surface and insuring a continued moist atmosphere by clamping a watch crystal over the surface. Three days after inoculation the epidermis was stripped off and mounted in lacto-phenol and Coton Bleu. The fungus stained a deep blue and thus the germ tubes could be traced readily upon the epidermis. Camera lucida drawings were made from the preparations. In such moist chamber growth the germ tubes are long and much branched, and run in all directions, as Weber (1922) has observed, without reference to the furrowed leaf surface. Weber reports, for the corn rust, stomatal entrances without, as well as with appressoria. According to my observations the lobed attachment disk over the stoma is typical (PLATE I, FIG. 1).

Infection in a vigorous host. The external phenomena of infection, as many times observed, are as follows: The first signs of infection visible to macroscopic observation are light green flecks in the green leaf. They are abundant five days after inoculation. Infections were successful with plants only one inch high with the leaves pushing out of the coleoptile. Attempts on several occasions to infect the coleoptile were unsuccessful. These attempts were not numerous enough to prove such infection impossible, but the rapid drying and early death of this tissue prevent any considerable development of the rust.

Pustules of uredospores are erumpent two or three days after the appearance of the flecks. These are soon surrounded by an oval border of secondary pustules at the circumference of the pale green spot. Later, in the case of vigorous infections, the sori become confluent in irregular patches which lie in yellowish or brownish areas in the green leaf. Teleutosori at their first appearance break the epidermis in the form of short clefts, as do

the uredosori, and they are distinguished among the uredosori only by the black coloring against the brown. They lie frequently along a vein, where the lines of sori tend to become confluent. Thus in late stages black streaks an inch or more in length are common, or crusts of close-lying, parallel, linear sori are formed. In the mature host, these aggregations of teleutosori occur chiefly along the midrib of the leaf, on the leaf sheath, and on the stem, both within and below the sheath. These tissues are similar to one another in their prominent vascular development. In these regions internal sori often lie directly below the external lines. In an earlier study (1924) I called attention to these internal sori as a probable expression of excess reproductive activity. These reproductive proliferations in a susceptible host are in contrast to an instance of an internal uredosorus reported by Allen (1926) for form 9 of *Puccinia graminis Tritici* on the resistant Khapli emmer. She interprets the case as an ineffective effort of a dying fungus to break through the host tissues.

There are of course many references in the literature to the occurrence of anthocyan in the tissue of infected plants, but the relation to its occurrence in uninfected tissue has been little discussed. My own observations merely add to the list of observed occurrences.

The presence of anthocyan was frequently observed in connection with infected regions of mature corn plants, especially in winter. In cases of scattered sori on vigorous green leaves anthocyan was sometimes present in a sharply defined border about each sorus or small group of sori. The purplish borders were often one-eighth of an inch broad around the sori. The secondary infections often lay in this border. This development of anthocyan is possibly a winter effect. The color faded as the sori ripened, and in late stages there were fewer examples of the sharply outlined red ovals, although anthocyan was present along the edges of the leaves and the midribs. In one case both uredo- and teleutosori had appeared on the leaf sheaths and even on the husks of a young ear, and these sori lay in the midst of irregular blotches of anthocyan. In another case, four-month-old corn plants showed anthocyan bordering sori on the older leaves and the leaf sheaths. In the most heavily infected leaves anthocyan was evident along the midrib, but not around the sori.

The anthocyan was naturally most sharply outlined around isolated primary infections, yet it is by no means so constant a feature at this stage as in heavily infected older plants, where it is less certainly related to the presence of the fungus. In cases of field infection with corn rust, anthocyan, while abundant in leaves and stems, appeared to have little significant relation to the rust.

Infection under inhibiting conditions: the so-called green island phenomenon. As widely observed and reported in the literature, under certain conditions the chlorophyll seems to be more persistent in the neighborhood of rust sori and some other fungus infections.

I regard these green spots as areas of balanced adjustment between host and fungus, as shown from a series of rust cultures upon subnormal corn plants. The first experiments deal with effects of over crowding of the plants. Corn was planted in seven-inch pots, five to seven seeds in a pot. When the seedlings were four weeks old and each had two or three leaves, they were sprayed with uredospores, and were incubated in a cold moist chamber. Observations were made eight days later. The five-week-old plants ranged from twelve to twenty-four inches high, and in every case had developed two or three additional leaves since the inoculation a week before. The plants were crowded in the seven-inch pots. This crowding had caused early dying of the older host leaves and thus a chance was given, even at such an early stage of infection, to observe the effects of death of the host tissue on the infection phenomena. There was a marked similarity in the leaf conditions on all the plants. The coleoptile had become a withered scale. The first leaf was either dry and brown, or yellow with a dry tip. The second leaf showed a dying tip. The third leaf and the two or three new leaves were green. The leaves present at the time of inoculation were in every case rusted, but the sori were usually limited to the terminal, that is, the older, half of the leaf. The lowest leaf had evidently dried too rapidly to allow much development of rust. There were scattered sori on this dead leaf and they were uniformly green-bordered. The second leaf showed green-bordered sori in its yellowed tip. The third and fourth leaves showed the heaviest infection. These were the terminal, somewhat inrolled leaves at the time of inoculation. They may have been more favorable

for inoculation because of their cornucopia-like form and hence their retention of moist air, but I believe their greater area and fresh condition were also favoring factors. The health of the leaf apparently favored the growth of the mycelium. The groups of sori on these fresh green leaves were surrounded by lighter green or by yellowish borders. The two upper leaves which had appeared since inoculation were in all cases uninfected.

Since all the sori on these plants at the time of observation (eight days after inoculation) were evidently the result of the single inoculation, we see here a definite variation in the behavior of the parasite, depending upon variations in condition of the leaves of the host. In the dying leaves the spread of the fungus is limited, but within the regions bordering the sori the death of the tissue is delayed, and the normal green appearance of the host tissue is long preserved, hence the phenomenon of 'green islands.' Just how the rust hyphae act to delay the death and drying out of the host tissue from which they are withdrawing both food and moisture is not at all clear. In fresh green leaves the clustered sori indicate a more extensive spread of the mycelium, and the bordering areas around the sori are paler in color as compared with the rest of the leaf.

The same contrast between infection in dying and in fresh tissue was often found within the limits of a single leaf, which showed 'green islands' on the dead or dying tip, and paler areas around the sori on the fresher, younger base of the leaf.

The effort to reproduce the colors in sketches of green-bordered sori leads me to think that the green appearing in the 'green islands' is as deep in color as in the normal leaf and does not appear green merely by contrast with adjacent yellow. If this is true, the spots must actually regain color, since they are deeper than the infection spot in the earlier, green leaf stage. Such a reaction indicates stimulation as well as preservative action by the fungus upon the dying host tissue. Allen (1926) thinks that this is what happens with the forms of *Puccinia graminis Tritici* which are able to effect some adjustment and live in a resistant host. She explains the pale fleck as due to a shrinking of the plastids, and the green spot as due to their subsequent enlargement. She finds the reversal chiefly at the borders of the infection spot, where the fungus in its advance effects apparently a better adjustment. She writes of form 27:

> When the hyphae in passing stop to form haustoria, the invaded cells often are stimulated, the cell contents become richer and the plastids larger . . . part or all of this outer zone regains a green color, and more rarely the central area also turns greener.

As I have noted in the review of literature, this is a phenomenon associated with degrees of resistance in the host, but Allen also notes the occurrence of 'green islands' under conditions that seem like those which I have described in the corn:

> When, as commonly happens, the infected leaf dies prematurely and the uninfected parts turn yellow, the areas occupied by mycelium still remain green and hold their chlorophyll more tenaciously than healthy tissues.

The same potbound conditions of corn were studied in a ten-inch pot of twenty plants which had become infected by standing near rusted stock. The plants had each developed six or seven leaves and rust sori were abundant. The contrast already noted between the lower dying leaves and the vigorous upper ones was apparent here. Under the potbound conditions, the lower two or three leaves on every plant were dead or dying. These showed green zones around the scattered sori as in the cases noted above. The upper leaves were still fairly vigorous. They showed heavy infection in all stages from flecks to abundant sori in light green areas. Later the heavily infected blotches became sere and dead, while the uninfected areas were green. Two plants in this pot had rotted at the base and their leaves were wilting at the time of the first observation. Here, as in the lower drying leaves of the other plants, the preservative action of the rust mycelium upon the host tissue was apparent. Even on the terminal leaves of these plants the areas around the sori stood out deep green in the wilted yellow-green leaf. The apparently mutualistic relations between host and parasite in the infected regions of these dying plants were in striking contrast to the progressive death of the host tissue as seen in other plants in the same pot, in areas of vigorous infection in a vigorous leaf. This retention of green in the rusted areas of a leaf dying from other causes was also strikingly shown in the case of a corn leaf which was kept in water in the laboratory for a week. When picked, the terminal half of the leaf was evenly green: a few groups of sori were just appearing and around these a faint yellow showed. Two days later the sori were more noticeable, and each sorus or group of sori was encircled by deep green while the

general leaf tissue was paler. By transmitted light four color areas were distinguishable: (1) a narrow light green ring immediately around the sorus; (2) a deep green ring; (3) a light area surrounding and often linking several groups of sori; (4) an intermediate green of the general leaf tissue. This differentiation of color zones around the sorus, in particular the occurrence of the deep green zone (2), seems to confirm the belief of Allen that there is a regaining of green in the outer region of infection. Four days later the upper third of the leaf had become dry and brown with 'green islands' still showing around the sori. Below this dry tip was an area which had become water-soaked. Here the general leaf tissue was transparent and colorless, but the sori stood out in the center of distinct 'green islands.' Anthocyan had developed both above and below the water-soaked area.

The 'green island' condition is less frequent in corn grown in the greenhouse during the summer. The continued spread of the infection produces more extensive dry yellow areas, following and including the areas with the light green bordering. The withered leaf shows no trace of green.

The same contrast between yellowed tissue around sori on normal vigorous leaves and green tissue around sori on less vigorous leaves is found in rusted corn growing out of doors. In September, 1921, a field of rusted Golden Bantam corn was found in Lenoxdale, Massachusetts. The field covered half an acre, stretching north and south in about ten rows. The infected plants occupied a square across the middle of the field in a slight hollow of the rolling ground. The infection seemed to have followed here much the same course as that described for the greenhouse stock. The stages are figured on plates 8 and 9. The most abundant sori were noticeably on fresh vigorous leaves. Around these sori the border was, according to age of the infection, either pale green, or dry and yellow. Figure 65 shows pale green borders around the sori in an early stage of infection in a fresh green leaf. Figure 66 shows dry areas around the confluent sori of a late stage of heavy infection in a green leaf. These conditions were found on the upper half of all the rusted plants. The lower leaves were in various stages of dryness and on them 'green islands' were frequent, and persistent long after the leaves were quite dry. Evidently the normal progressive dying off of the lower leaves in this thickly planted field produced

the same effect as that observed in the potbound greenhouse plants. Figure 67 shows the green-bordered sori in one of these dying leaves which is yellowing but still succulent. Figures 68 and 69 show the green bordered sori in nearly dry basal leaves. Figure 70 shows a few persistent 'green islands' in a dry, brown, basal leaf. This figure indicates also, by the small number of its sori, the check which the rapid drying of the basal leaves offers to the spread of infection. On these dry leaves the sori had frequently not broken the epidermis. This is perhaps another evidence that the growth of the parasite was checked by the weakening of the host. Corn in this field had made a fairly normal development, notwithstanding the rust. The ears were well-filled and apparently as abundant as in the unrusted part of the field. The host growth had generally outstripped that of the fungus, as the upper leaves were but slightly infected, and the leaves of the rather numerous axillary branches and the husks were seldom infected. The heaviest infections were usually on the fourth or fifth leaves from the top. Frequently the infection stopped abruptly here. One low plant whose top leaf was on a level with the third of the next plant was completely rusted, the second leaf from the top showing the most numerous sori.

An extreme case of heavy rust infection was found in October, 1923, in a field of Golden Bantam corn in Norton, Massachusetts. Practically every leaf on the plants was dusty with erumpent sori of both uredo- and teleutospores. The latter in particular made heavy crusts on leaf bases, leaf sheaths, and stems. Dehiscence of the sori on the inner surface was a frequent occurrence in the case of the leaf sheaths, and thus stem infection had resulted. The rust growth had kept pace with the growth of the host, extending even into husks and tassels. The dehiscence of the sori on the husks was often on their inner surfaces and by this means there had apparently resulted a progressive infection through several layers. Infection of the kernels was not observed. In the tassel both uredo- and teleutosori were abundant on each rachis and pedicel and on the walls of the anther sacs. Several plants showed smut galls as well as rust. The farmer reported the ears from this rusted corn abundant and of good quality, but said they had run small and were not evenly filled out. He had made three plantings at successive dates and all three had become in-

fected. In this instance of excessive infection on vigorous host plants there was comparatively little expression of the 'green island' phenomenon. Instead, the borders around individual sori or around the blotches of confluent sori were usually dry and brown in the green leaves. Only on occasional dry basal leaves did the green spots show around sori. In these cases the conditions of the potbound plants were evidently repeated. Green spots were abundant on a branch above a basal leaf on a dry plant of the first planting. This shoot was evidently of later growth than the main plant and was dying from a rotted base. Here again, in the ill-health of the host the preservative action of the fungus appeared.

'Green island' formation was also observed in the greenhouse in the case of *Uromyces appendiculatus* on seedlings of Kentucky Wonder Bean, which had been inoculated when two weeks old with spores from infected bean pods. A month later the plants showed both uredo- and teleutosori. As in the corn, these were bordered with green on the dying older leaves. In this netted-veined leaf the infected spots are circular, while in the corn they are oval or oblong between the parallel veins. Secondary sori were formed at the circumference of the green area. In the dry leaf an outer border of more transparent tissue appeared around the green spot.

Cytological phenomena of infection

The haustorium and its relations to the host cell. Cytological studies of infected corn tissue were made from a variety of fixations. The solutions of Flemming, Bouin, Merkel, and Carnoy, and 50 per cent alcohol were used. Flemming's medium solution was the fixative most frequently used. Flemming's triple stain was most frequently used, but comparisons were made with Gram, Gram-Weigert, and Heidenhain's haematoxylin.

Plates 1 to 3 show the haustorium in its relations to the host cell in leaves whose stages represent the normal course of a vigorous infection. Figures 2 to 13, plate 1, are from leaves which showed merely the pale fleck, the first sign of infection. Spores lie on the surface of the leaf and germ tubes are visible. Substomatal vesicles occur, whose proliferations reach the opposite epidermis. Young haustoria are abundant in both mesophyll and epidermal cells. The haustoria arise usually from a densely

filled, terminal, hyphal cell, which appresses itself to the host cell wall (FIG. 2). Sometimes this haustorium mother cell is a branch from a terminal cell (FIG. 10). Occasionally it is the penultimate, instead of the terminal cell (FIG. 9). Its paired nuclei are usually clearly marked (FIG. 11).

Parenchymatous cells of the type commonly attacked by the haustoria of rusts are in general turgid vesicles bounded by a wall and plasma membrane, and with a large central vacuole filled with a sap, whose osmotic relations with its environment are commonly assumed to determine the turgidity of the cell. I shall use the old term primordial utricle for the peripheral layer of the protoplast, including both plasma membrane and tonoplast. The question whether the penetration of such a cell by a fungous hypha or a haustorium involves the puncturing of this primordial utricle with release of the cell sap, or merely its invagination, has been raised by many students of host-parasite relations.

The corn rust haustorium plainly pushes in the primordial utricle rather than pierces it. This fact is particularly evident in these early stages. There is no apparent thickening or alteration in the host cell wall at the place of contact, and no invagination of the wall. The penetrating filament arises as a mere papilla, and develops as what appears like a very slender rod of densely staining homogeneous substance. There is a clear area between this minute peg and the primordial utricle (FIGS. 3, 4a). This appears also around the dark spherical tip shown in figures 5 and 6, and around the lobed tip of figure 7. Apparently this area later becomes the digestion cavity, which may be seen more or less clearly around lobes of the haustoria.

The characteristic lobing of the haustorium commences while the contents, with the methods used, still stain as an undifferentiated mass. Most frequently the terminal knob becomes irregular and forms two or more lobes, each of which in the mature form has one or more nuclei. Figures 7 to 12 illustrate the lobing. Occasionally the branching commences midway on the filament, leaving the stalked knob as the central branch (FIGS. 8, 12). Occasionally one finds an unbranched haustorium which has enlarged into a nucleate vesicle. The lobed haustoria must develop rather rapidly, as mature forms are not infrequent in this flecked leaf material. Figure 13 shows the characteristic

short, slender stalk and digitate lobes of the mature haustorium. The dense cytoplasmic contents and the well defined nuclei indicate activity of some kind in the haustorium. It is perhaps significant that the nuclei lie in the larger lobe, which indents the host cell nucleus. The fungous cytoplasm is especially dense at the tip of this lobe. There are frequent contacts, in this material, between haustorium and host cell nucleus, but they occur usually with the more advanced haustoria. I did not observe cases where the host cell nucleus lay at the point of penetration. Both fungus and host cells appear to be in healthy condition. Chloroplasts in the primordial utricle are of the same size as in uninfected cells. The host cell nucleus is of normal size and shows a delicate reticulum.

Figures 14 to 18, plate 2, show slightly older stages of the rust, from a leaf which showed young sori surrounded by pale green in a green leaf. Within these spots the fixed material shows that the mycelium crowds all the intercellular spaces. Practically every mesophyll cell and many epidermal cells are penetrated by haustoria. The short-stalked, digitately lobed haustorium is seen here in great variety. The two-lobed form is at first sight most common, but it often proves to have other lobes lying in a different optical plane. The three-lobed haustorium shown in figure 16 is in reality five-lobed, and the one shown in figure 18 is four-lobed. A single, auricled sac is frequent in epidermal cells (FIG. 17).

In the stalks of these haustoria, as compared with those from the flecked leaves, one finds a development which may, I think, serve as an index of a change in the host-parasite relation. Figure 15 shows one haustorium with a filiform stalk such as has already been described as typical of the mature form. The second haustorium in the cell shows a conspicuous thickening half way up the stalk. Figure 14 shows a slight thickening on the stalk. Figure 18 shows a more decided basal ring. Throughout the sections of this material, along with many slender-stalked haustoria, there are many variations of a broad or narrow ring-like zone about the stalk. It is not at all clear whether this thickening arises as a differentiation of the outer surface of the haustorial wall or as a deposit from the host cytoplasm. Its position on the stalk, however, frequently coincides with a layer of host cytoplasm (FIG. 14).

In both the haustoria of figure 14 a thin wall is distinguishable within the so-called digestion vacuole. In this, as well as in the case of other lobed haustoria figured later, the clear enclosing area cannot be traced completely around the lobes, but its occurrence indicates digestive activity on the part of the haustorium, and offers evidence for invagination of the host cytoplasm. Figure 14 shows a rare case of a host nucleus appressed against the base of the haustorial lobes. Allen (1926) finds such a position a frequent occurrence in rusted Khapli emmer, and sees in it a host-nuclear offensive on the part of this resistant wheat.

Figures 19 and 20, plate 2, and figures 24 to 31, plate 3, show conditions in later stages of the corn rust, when erumpent pustules lie in yellowed areas in a green leaf. The material from which figures 19, 20, and 24 to 29 were taken was fixed in Flemming's stronger solution. Possibly on this account the host cells show evidences of disorganization. Note the possible excretion products and the plasmolysis in the cell of figure 19; note also the clumping and shrinking of plastids and disorganization of host cell nucleus in figure 24. The sections were cut longitudinally. In figures 24 and 25 a crowded mycelium is noticeable in the intercellular spaces which make re-entrant angles into the lobed mesophyll cells. Haustoria frequently arise from these intrusions and extend parallel to the long axis of the cell (FIG. 25). Figures 19 and 26 show clear-cut digestion vacuoles.

The series was chosen to show variations in the haustorial thickenings. Some degree of thickening is characteristic of practically all haustoria in this material. They stain regularly like the thickenings of the host walls. They are most often deep blue or purple with the triple stain, but in some cases they take an excess of orange-G. In the median optical section of figure 19 the protein core of the haustorium shows within the thickened stalk; in figure 20 the contours of the heavy wall of the haustorium suggest lobes within the sheath. The basal socket (FIGS. 25, 26) is so conspicuous in this material that it often serves to locate haustoria whose lobes extend into a different plane. The stalked cup is at times found broken off in the cell, and may lie so that the lumen of the haustorial tube shows as a circular opening. Very commonly the thickening forms a cup at the base of the lobes of the haustorium instead of around the stalk (FIGS. 24, 27). Figures 28 and 29 show a condition, frequent in

epidermal cells, of haustoria which fail to develop the normal stalked form, and become completely encased. Figure 28 shows projections of host cytoplasm around the knob-shaped mass. Figure 29 shows an invaginated plasma membrane of the host cell. The conjugate nuclei within the heavy wall in figure 29 indicate that the haustorium was still alive. These epidermal cells are, I think, the last to be invaded, since the hyphal branches from the substomatal vesicle spread more rapidly through the mesophyll. Possibly the fungus is by this time less vigorous; possibly the greater thickness of epidermal cell walls is correlated with sheath formation. The heaviest sheathing is in general found where a haustorium has entered a thick-walled cell. Thus the cells immediately under a sorus are most apt to contain heavily sheathed haustoria. Allen (1926) thinks that the epidermal cells of Khapli emmer differ chemically from the rest of the leaf. In this resistant wheat, however, the reaction which she reports is the reverse of what I have found in corn, where the epidermal cells show less resistance to rust.

Figures 30 and 31 are cross sections from different leaves which were, however, at the same infection stage as the rest of those shown in plate 3. In figure 30 thickenings have developed on the stalks of both haustoria. In figure 31 the haustorial lobes not only issue from a lobed socket, but are apparently thick-walled over their whole extent, as they stain deep orange; nuclei are discernible in the lobes; an hypertrophied host cell nucleus overlies the lobes. This haustorium seems a massive development in contrast to a second one in the cell. This second one is a slender-stalked, lobed form, typical of earlier stages of the rust. Its walls are evidently thin, as the purple of the protoplasmic contents is its dominant color.

I found evidence of an interrelation between sheathing of haustoria and a heavy drain upon the host incident to a heavy infection, in material from the rusted Golden Bantam corn from Norton, whose extreme infection I have already described. Many of the leaves from these plants were covered on both sides with sori, and there were many instances of internal sori. These cases of internal sori furnish additional data in support of my conclusion (1924) that the phenomenon of internal sori is due to excessive reproductive activity.

Figures 32 to 34, plate 3, show cells from a heavily infected

leaf sheath of this corn. The sori lay in yellow areas in a green leaf. Sori of teleutospores are confluent on both surfaces and between these the leaf is double the normal width by reason of crowded cysts of internal sori. In addition to the intracellular sori the intercellular hyphal masses form a pseudoparenchyma. The hyphae are poor in contents and stain deep orange, making a striking contrast to the purple or blue of the thick host cell walls. Both the orange-stained host cytoplasm and the red-stained nuclei show disorganization, but this may be due in part to heavy frosts, which occurred before I gathered the leaves. The haustoria in this tissue all show some degree of sheathing. The sheaths are exceptionally heavy in the thick-walled cells in the vicinity of sori. The haustorial thickenings show the same staining reaction and the same layered effect as the host cell walls. Figures 32 and 33 show haustorial lobes issuing from heavy basal sockets. Figure 34, from a tangential section, shows the contrast between the thinner walls and less developed sheaths at a distance from a sorus, and the thick walls and thick sheaths near a sorus.

A further suggestion of a correlation between haustorial thickening and the virulence of the fungus is indicated in a series of sections of leaf sheaths and of stem and cob tissues from infected corn grown in the greenhouse, which contrast the conditions around uredosori in green tissue with those around teleutosori in yellowing tissue. Figures 35 to 37, plate 4, are from uredo material; figures 38 to 40 are from teleuto material. Cells in the region of the young uredosori show abundant mature haustoria. They are many-lobed, and usually in close contact with the host nucleus. The plastids are frequently clumped around nucleus and haustoria. The nucleus is often hypertrophied, as may be seen by comparison of figure 35, an infected cell, with figure 36, an uninfected cell. With all this evidence of abnormal conditions due to the presence of the parasite, the haustoria in this fresh green tissue are uniformly slender-stalked. Figure 37, from the tissue of a young corn cob which was artificially infected, shows an exception. This thick-walled cell was isolated by hyphae of a sorus. The walls of host cell and of haustorium are shaded in the figure. In general, throughout the soft thin-walled parenchyma of the cob the slender-stalked form of haustorium prevails. Haustorial sheaths occur in connection with the heavier demands

incident to teleutospore production. Figure 39 is from heavily infected tissue of a leaf sheath below a teleutosorus. There is extreme hypertrophy of both host cell and host nucleus. The haustorium issues from a well defined basal socket and indents the nucleus in the midst of a tangled mass of cytoplasm, plastids, and haustorial lobes. Figures 38 and 40 are from a section of a leaf bearing teleutospores. A long teleutosorus lay along the midrib and beneath this an internal sorus had developed. It was therefore an extreme case of heavy teleutospore production. The intercellular spaces are packed with mycelium and one or more haustoria are in almost every cell. Yet only in the thick-walled cells near the sori are haustoria with thickened walls found (FIG. 38). In the thin-walled inner parenchyma of the storage tissue around the midrib the numerous haustoria are all slender-stalked forms with no thickening (FIG. 40). Figure 40 is a good illustration of the typical nuclear condition of haustoria of the corn rust: the haustorium is multinucleate; the nuclei occur in conjugate form, agreeing with the expected condition for uredo-teleuto mycelium. Such median optical views as that of the haustorium in figure 39 show nuclei in the basal part as well as in the lobes.

In all these cases figured in plates 2 to 4, the thickening of the haustorial boundary, whether by a deposit of the host cell or not, seems to be a phenomenon correlated with the yellowing tissue and the much thickened host walls. The existence of such a correlation is confirmed by the comparison of haustoria from 'green island' tissue with haustoria from yellowed infection areas in a green leaf, the normal condition of advanced infection. Haustoria from the yellowed areas have been described, and are figured on plate 3. The difference between these sheathed forms and the haustoria in green tissue is strikingly shown in figures from a leaf strip which extended across a yellowed patch bearing two uredosori into green leaf tissue (see plate 5). In the green tissue no mycelium is found, and the plastids of the bundle sheaths show starch grains. In the yellowed tissue no starch shows, the mycelium is abundant, and there are haustoria in most of the cells. Figures 41 to 43 show cells from the edge of the yellowed tissue near the green. The cells are surrounded by abundant mycelium and are hypertrophied, but the haustoria are seldom sheathed. The remaining figures show cells from

the yellowed tissue. The haustoria here are of the sheathed types already figured as typical of advanced infection. In figure 44 a so-called digestion vacuole shows clearly between the apparently dead, deeply stained haustorium and the dense accumulation of host cytoplasm around it. This is a thick-walled cell near a sorus. In figure 45 thickenings are shown on the stalk of a young haustorium in an epidermal cell. In figure 46 secondary thickenings are evident in a thick-walled cell of a bundle. The basal socket of the haustorium takes the same deep stain as the secondary cell thickenings, while the thickening which nearly covers the lobes is stained a paler purple. In figure 47 the cell walls are thickened at the places of penetration of two haustoria. In one case the wall thickening evidently kept pace for a time with the penetrating filament. The haustorium projects from a conical thickening which is distinctly laminated. The cells of this figure are beyond the sorus near a region of dead cells which lies between two seri. The cytoplasm in these dead cells is contracted around dense red-stained haustoria. Thus in this section one may trace a progression from slender-stalked haustoria in thin-walled cells near the green host tissue, to thickenings around haustoria and around intercellular hyphae in regions of thick-walled cells, and finally to dead haustoria in dead host cells. The dead area is limited in extent, lying between two infected areas.

Cells from the green-bordered sori are shown in figures 21 to 23, plate 2. Their haustoria agree in type with those on the same plate taken from leaves of less advanced infection. The intercellular spaces of this tissue are crowded with hyphae, and haustoria are numerous. They catch the eye even under low power, as they stain deep purple with the triple stain, while the host tissue and the intercellular hyphae, except for terminal cells, stain orange. Occasionally a terminal hyphal cell or a haustorium stains red. Occasionally a haustorium stains orange with a red nucleolus like the host nucleus. In figure 22 the upper haustorium is stained like the host nucleus, while the other two haustoria in the cell are deep purple. The striking thing about all the haustoria from these heavily infected green areas is the slender stalk. There is no trace of sheathing; yet, judging from the ripe sorus which lay near the epidermal cell of figure 23 and the abundant intercellular mycelium shown in

figure 21, this tissue is in an advanced stage of infection. The uninfected cells beyond the green areas are poor in cell contents with faintly stained plastids. This agrees with the observation of Mains (1917) that the infected cells of a green spot draw upon the surrounding uninfected cells until, under the influence of the rust, the infected cells become parasitic upon the uninfected.

In addition to thickenings around haustoria and thickened walls of infected cells, I have found in corn leaves heavy, deeply stained walls in groups of cells near the limits of an infection area. In the leaf section described in connection with plate 5, these deeply stained cell groups stand out in the regions between the sori. In these same regions there are many dead cells with collapsed walls and plasmolyzed contents around dense, red-stained haustoria.

The appearance of thickened host cell walls in such cases is not easily explained, and, so far as I have found, has been noted only a few times in the literature. The staining tests which I have made do not determine definitely whether the deeply stained walls show secondary thickening or are gelatinized. The staining reactions are generally like those of thickened host walls.

These thickened spots suggest the condition which Magnus (1897) describes as caused by *Aecidium Magellanicum* in the barberry. The fungus causes a general stimulation of the tissues, resulting in witches broom formations, and also in a local toxic action upon the infected cortical cells of these shoots. The cells between which the hyphae run, and in which they form knotted haustoria, develop abnormally thick brown walls until the groups lie like islands in the midst of thin-walled tissue. Conversely, the mycelium seems to be injured by this contact. Frequently these thickened cells, together with the dead brown hyphae and the knotted haustoria (a pathologic group according to Magnus), are cut off by a ring of cork. Allen (1923) calls attention to increased thickness in host walls as a possible evidence of resistance by Kanred wheat to toxic secretions of rust. The thickenings which she describes, however, were between dead cells and normal ones and were least developed at the intercellular spaces, the usual place of entrance for haustoria. They appear to be much less pronounced than those I have found in corn. These deeply stained cell groups in the corn seem very different also

from the swelling of the host cell walls which Allen (1923) describes as a secondary effect of *Puccinia graminis Tritici*, form 3, in resistant Mindum wheat. She states, 'These swollen walls take no stain (with the triple stain at least) and are transparent . . . such a wall must be almost lignified.' Dickson, Eckerson, and Link (1923) think that the factor which checks the penetration of corn and wheat seedlings by *Gibberella Saubinetii* is a maturation change in the cell membranes of the cells of the cotyledon sheath. At the outset, they state, these sheaths are composed of easily penetrable pectin. At maturity cellulose or even lignin and suberin predominate 'and these less easily hydrolyzable wall elements resist the invasion of the fungus.' Holbert and Koehler (1924) report thickened walls of pith and cortical cells of roots to be a distinctive character of certain strains of corn which are resistant to rootrot and smut.

The figures shown in plates 1 to 5 were selected with the purpose of comparing haustorial development under varying conditions of host tissues. I have called attention in several figures to instances of contacts between haustorium and host nucleus. A summary of conditions concerning contact as shown in these plates should, I think, give the typical habit for the corn rust, inasmuch as the figures were chosen by chance, so far as the matter of contact is concerned.

I find that the haustorium is very frequently in contact with the host nucleus. This does not seem to me to be due necessarily to specific action on the part of the host or the parasite. I find no evidence of an approach to the haustorium by the host nucleus. The nucleus is not generally found at the penetration spot of the young haustorium, as Allen (1926) finds it in infected cells of resistant wheat. It is in other positions or not in view in the figures of young haustoria (PLATE 1). Figure 12, plate 1, is an exception, in showing the nucleus near the young haustorium. The host nucleus is rarely found near the haustorial stalk or near the origin of the haustorial lobes. Figure 14, plate 2, and figure 26, plate 3, are the only examples of the nucleus in this position which I have noticed. The host nucleus certainly does not appear to seek out the growing point of the haustorium.

My figures show that the host nucleus is not infrequently more or less in contact with the lobes of the mature haustorium (FIG. 31, PLATE 3). It may more rarely be enwrapped by the

haustorial lobes. In many cases the contact between the nucleus and a haustorial lobe is so close that the nucleus may be said to be amoeboid and to enwrap the haustorium, or the haustorial lobes may be said to be indenting the nucleus (FIG. 13, PLATE 1; FIG. 39, PLATE 4). Such situations as those shown in figure 22, plate 2, and figure 35, plate 4, where haustoria from different sides of the cell converge upon the host nucleus, suggest strongly that the position is due to more than chance, and suggest a positive tropism of the haustorium in relation to the nucleus.

These contacts seem as frequent in fresh green corn tissue as in the yellowed tissue. In the latter, however, there are more evidences in the nuclei of abnormality due to the contacts. In figure 13, plate 1, the host nucleus, although indented, is normal in size and contents. The nucleus of an uninfected cell from this section may be compared in figure 2, plate 1. These preparations are taken from material which had not yet produced spores. Even in material with erumpent sori the nuclei are, as a rule, only slightly hypertrophied. The strongly hypertrophied nuclei shown in figures 35 and 39, plate 4, are rare in corn. These were found in heavily rusted leaf-sheath material.

Of seventy-five sketches made from various tissues of corn, and at various histogenetic stages, thirty-three show the host nucleus in the vicinity of the haustorium. A count of nuclear contacts was made from the material sketched in plate 3; from twenty-seven microscopic fields, averaging twenty cells to a field, fifty-six haustoria were counted without the host nucleus in view, and sixty-three with the host nucleus showing. Of the latter, all but two showed contact between nucleus and haustorium. The low average of only four out of twenty cells to a field which show haustoria does not express the degree of infection for this material, as the many cells containing only cross sections of haustorial lobes were omitted. These cells were considered indeterminate with respect to the relative positions of the host nucleus and the haustorium.

Effects of rust on reserve carbohydrates

Attempts to determine food conditions in the leaf of the corn host require a recognition of certain anatomical and physiological characters of the corn. Stevens (1916) states the facts briefly and clearly:

> In Indian corn each of the veinlets is surrounded by a sheath of border parenchyma cells and these in turn by palisade cells radiating from them. . . . In corn, as indeed in many grasses, starch is not formed in the palisade cells at all but the parenchyma sheath on bright days is filled with it. . . . Of course the palisade cells manufacture soluble carbohydrate abundantly and the starch in the sheath cells doubtless represents a surplus that comes to the sheath faster than it can be conducted away.

This localization of the starch storage is proved by the iodine test. Corn leaves treated with iodine darken only along the bundle lines. Healthy leaves were used as a control for tests of rusted leaves. When infected leaves which showed a yellowed zone around the rust sori were treated with iodine, the darkened lines of the bundles were interrupted in these regions. Starch was evidently not present in the bundle sheaths in these yellowed zones. This agrees with the results reported by Mains for stages of rusting when the drain upon the host becomes so heavy as to prevent starch storage.

The sheath cells of the bundle in the corn leaf seem to be more crowded with plastids than the mesophyll cells, and their plastids seem less quickly subject to disorganization. Pale leaves striped with green along the bundles are a familiar phenomenon in a corn field. Apparently this may result from a variety of causes, but the chlorophyll seems regularly more persistent in the bundle sheaths. When normal leaf tissue is stained with the triple stain, the plastids in the bundle sheath cells stain deep red, while those of the mesophyll stain much paler. In rust-infected areas this tendency of the plastids of the sheath cells to stain deeply is lost. They stain the same pale red or yellow as do those in the surrounding mesophyll cells. The loss of chlorophyll, the external sign of infection, thus seems to correlate with lessened staining capacity in the plastids of infected areas. This reaction is not dependent upon the presence of haustoria in the sheath cells themselves. Even in advanced infections the sheath cells are seldom invaded. The mesophyll may be crowded with intercellular hyphae and with haustoria, but the fungus usually stops abruptly at the sheath circle. Figure 46, plate 5, shows an exceptional case of a haustorium in the parenchyma of a bundle. Mains (1917) states of the corn rust, 'The vascular bundles are apparently never invaded by the mycelium and the parenchyma sheaths which surround them only occasionally have haustoria in their cells.' Mains also notes the indirect effect of the rust

upon the uninvaded sheath cells, 'Since the parenchyma sheaths serve as a storehouse for the assimilated material from the adjacent region, and since they are not invaded for some time, it would appear that this loss of starch is due, not to a withdrawal of starch from the parenchyma sheath by the fungus itself, but to the utilization by the fungus of the material formed in the neighboring region before it reaches the parenchyma sheaths.'

My best results in differentiating starch in the chloroplasts of the corn leaf were obtained with fixation in 50 per cent alcohol, followed by the triple stain. This was the preparation of the material used for the figures of plate 5. The mature rust sori of this material lay in a yellow area, but one end of the section extended into green tissue. It is in this end only that starch is shown in the bundle sheath cells. The starch grains are stained pale blue, and the plastids yellow. No starch is differentiated in the plastids of the mesophyll cells.

Haustoria of other rusts

I have studied for comparison, but in less detail, the haustoria of several other rusts. The forms illustrate the first three types given in Arnaud's classification of haustoria: the simple, the spiral, and the digitate. His fourth type, the coralloid haustorium, which is so abundant in the Asterineae, I have not yet found in the Uredineae.

Puccinia claytoniata Peck. Plants of *Claytonia virginiana* heavily rusted with *Puccinia claytoniata* were gathered in May, 1922. The rusted leaves are slightly thicker than the normal leaf. Spermogonia and aecidia were both erumpent. Some yellowed leaves were crowded with aecidia on both surfaces. Other leaves showed only spermogonia and were still green. Figures 48 and 49, plate 6, show cells from this younger, green material. The tissue shows a heavy infection, with spermogonia just breaking the epidermis. In their vicinity the hyphae make a felted mass. Throughout the leaf, hyphae crowd the intercellular spaces. Unlike the corn rust, they invade the intercellular spaces of the vascular bundles, and send haustoria into the wood parenchyma cells, and even into the vessels. The haustoria are elongated vesicles, bent or twisted, but for the most part unbranched. Only occasional encased forms in epidermal cells resemble the short, knob-like forms described by Fromme (1914)

for the same rust. Figure 48 shows a haustorium overlying a lobed host nucleus and surrounded by cytoplasm and plastids. Lobing and hypertrophy of the host nucleus and contact between host nucleus and haustorium are more frequent in the infected *Claytonia* tissue than in the case of the corn rust. The cytoplasm of the host cells is regularly set with plastids close to the wall. Their position in figure 48 indicates invagination. The massing of cytoplasm around the haustorium seems evidence that cell activities center in the vicinity of the haustorium. In figure 49 a binucleate haustorium is enclosed in dense layers of cytoplasm. Cytoplasm frequently makes an irregular coating over the heavily encased knob-like haustoria of epidermal cells. These conditions agree with that described by Allen (1923) as a reaction of Mindum wheat to *Puccinia graminis Tritici* and support her suggestion that the haustorial sheath originates from host cytoplasm. All degrees of sheathing are found in the infected *Claytonia* tissue, and its occurrence is always coincident with some degree of thickening of the host cell walls. The mesophyll cell of *Claytonia* is normally thin-walled. In figure 48 the haustorium issues from a basal socket which seems continuous with the thickened wall of the host cell. Both are shown in black in the figure. In figure 49 the host cell wall is much thickened at the point where the haustorium entered. This may be a swelling due to parasitic action, but similarity in staining reactions indicates strongly the common origin of this host cell wall and the haustorial sheath.

Puccinia Violae DC. Figures 50 and 51, plate 6, are from leaves of *Viola cucullata* infected with the uredo stage of *Puccinia Violae*. Mycelium was abundant throughout the leaf tissue, but the sori were overrun with an infection of *Darluca filum* which had fruited abundantly and formed dense stromata at the base of the sori. I did not find haustoria of *Darluca*. The hyphae of *Darluca* are distinguished by their smaller nuclei, and they are closely appressed to hyphae of the rust. The haustoria of the rust may issue from a basal socket, or they may be completely encased in a thick wall (FIG. 50), or they may have merely a thickened tip (FIG. 51). Those with thickened tips appear similar to the ones described by Colley (1918) for *Cronartium ribicola*. Many of the violet cells are nearly filled with a yellow-staining homogeneous substance. Figure 50 shows this indented by the haustorium. The small host nucleus is shown in figure 51. This

is typical both in its faint staining reaction and in the lack of differentiation of a nucleolus. It is not typical, however, in its nearness to the haustorium. The haustorium is only infrequently found in the vicinity of the host cell nucleus. The haustoria are usually binucleate. The two figured have triple nuclei. These are frequent enough in the material to indicate that the haustorium does not necessarily have the same number of nuclei as ordinary hyphal cells.

Aecidium punctatum Pers. In the fall of 1923 three plants of *Hepatica acutiloba* were transplanted from the vicinity of Pittsfield, Massachusetts, to Norton, where *Hepatica* does not occur. In the spring of 1924 one of the group developed rust upon its new leaves. This same plant developed rust in 1925, while the other two plants in the group remained uninfected. In 1926 the rust developed also on another of the three plants.

Accidium punctatum is very favorable material for the study of haustoria. In the infected area around a sorus, haustoria occur in almost every epidermal cell, as well as in the mesophyll cells. Figure 52, plate 6, shows a haustorium in a mesophyll cell. Although the leaf was completely dotted with spermogonia and was altered into a smooth erect form, it was fresh and green. The cell figured shows apparently healthy conditions of cytoplasm, plastids, and nucleus. The haustorium coils abruptly after entering the cell, and then extends a twisted lobe into the vicinity of the host nucleus. The base of the haustorium seems more thickly walled than the lobe. There is great uniformity in haustorial form and habit throughout the infected tissue.

Puccinia Asterum Kern. Figure 53 is from a section of a leaf of *Solidago* sp. infected with *Puccinia Asterum*. This is another case in which the mutualistic relation between host and rust is obvious. The only indication of any abnormality in the host cell is the presence of red-stained bodies which are possibly waste products. The cells of this leaf are fairly thick walled; yet there is no indication of any sheath around the haustorium. A well defined clear area appears around both the longitudinal and cross sections of the haustoria. Both of the lobes are in close contact with the host nucleus. The binucleate haustorium is of interest on this aecidium-bearing mycelium.

Uromyces appendiculatus Fries. Figure 54 shows a simple sac-shaped haustorium from an infected leaf of Kentucky Wonder

Bean. The slender, stalked vesicle, with its vacuolated cytoplasm and reticulated nucleus, is well differentiated. The host cytoplasm and plastids are massed in the vicinity of the haustorium. The plastids in the tissue below the sorus, the region of the cell figured, are contracted and are stained pale yellow. In the tissue on either side of the sorus the plastids stain bright red.

Uromyces houstoniatus Schweinitz. This species is apparently perennial in *Houstonia caerulea*. Infected plants are etiolated, and are noticeable in the field because of their pale yellow color and greater height. Stems, leaves, and buds showed erumpent spermogonia and aecidia. Material was fixed in Flemming's medium solution in May, 1923. The mycelium is abundant in the intercellular spaces of all these tissues, forming runners in the parenchyma and the bundle sheaths of stem tissue, and entwining the cells of growing points and leaves. The haustoria are long hypha-like diverticula, occasionally branched, always much curved and twisted so that it is difficult to get a complete view of one. They frequently enwrap the host cell nucleus. In epidermal cells near the spermogonia, and in bundle sheath cells, the haustoria are at times thick-walled, but in the majority of cases they show no thickenings. Figure 55 shows a cell from leaf tissue. This haustorium is unusual in having a branch. Its cytoplasm also shows an abrupt change from a deep blue stain up to the origin of the branch and a pale stain in the terminal half. What G. Smith (1900) called a digestion vacuole is well defined around the base of the haustorium. The tip of the haustorium evidently curves into another plane.

Uromyces Caladii Farlow. I have studied a plot of rust-infected *Arisaema* for three seasons at Norton. Spermogonia and aecidia develop each May upon the unfolding leaves and floral parts. As a rule even heavily rusted leaves and spathes develop rather normally. All parts of the *Arisaema* shoots are heavily infected with the rust, the intercellular spaces being stuffed with mycelium. Several corms of infected plants were fixed and sectioned but no trace of the mycelium of the fungus was found. More work is needed to determine whether this rust 'repeats' by means of an annual infection from spores in the soil, instead of living as a perennial mycelium. My negative findings at least suggest the possibility that this is another case like that found by Whetzel, Jackson and Mains (1924) for the rust on *Podophyllum*.

The infection appears, however, typically systemic. The haustoria show helix-like coils in close contact with the host nucleus. These conditions are shown in figure 56, a cell from the layer just below the columnar epidermis of the smooth tip of a spadix. In the section almost every cell of this layer showed one or more haustoria. The spongy tissue within was also heavily infected. The epidermal layer had few haustoria, although spermogonia dotted its surface. The rust grows with great luxuriance in the soft succulent tissue of *Arisaema*. In certain prepared sections of the stem where the host walls happened to stain very faintly, the pattern of the host cells appeared outlined by the fungus. The hyphae encircle every cell, and haustoria penetrate from each intercellular space. It is to be noted that the thin walls of the host tissue correlate with the general absence of thickened boundaries around the haustoria. Only in cells near spermogonia was an occasional instance found of a haustorium with thickened boundary.

Aecidium Sambuci Schweinitz. *Sambucus canadensis* was found in Norton in June, 1922, heavily infected with *Aecidium Sambuci*. The rust formed yellow galls on the young stems, the petioles, and the mid-ribs of leaflets. An intercellular mycelium is abundant in these galls. Slender, deeply stained hyphae encircle the hypertrophied cells (FIG. 58, PLATE 7). Almost every cell throughout the gall is penetrated by haustoria. The cells of the bundle sheath are frequently entered (FIG. 57). The haustoria are ribbon-like (FIG. 58), or arborescent (FIG. 57). More rarely the slender-stalked sac typical of the corn rust is found. There is an appearance of activity in the cells under the stimulus of the fungus. Hyperplasia is common in the host cells. Figure 58 is an example, showing a haustorium in contact with a host nucleus in prophase.

Chrysomyxa Pyrolae Rostr. *Chrysomyxa Pyrolae* occurs in Norton, Massachusetts, in the spring on the overwintered leaves of *Pyrola americana*. The orange uredosori appear in early May, covering the under surface of the leaves. About two weeks later the paler yellow teleutosori appear in scattered groups among the uredosori. The habit of this rust makes an interesting contrast to *Aecidium punctatum*, which I have described above. *Chrysomyxa Pyrolae* apparently overwinters in the rootstock and terminal bud of the host; the uredo and teleutospores

appear on the leaves, usually, only in their second year. *Aecidium punctatum* seems, from its recurrence on the *Hepatica* plant studied, to be also a perennial rust, but its spermogonia and aecidia develop on the spring leaves as they unfold.

Pyrola leaves bearing uredosori only are well supplied with starch. The large storage grains are massed in the mesophyll cells. They are enclosed in the cytoplasm and are often crowded to the wall by a dense homogeneous substance which fills the vacuole. This seemingly mucilaginous substance occurs throughout the mesophyll. It is of similar appearance to the vacuolar content already noted in the cells of *Viola cucullata*. It stains greenish yellow with the triple stain and with the Gram-Weigert stain. Figure 60 shows the starch grains lying upon the periphery of the dense yellow substance in the primordial utricle. A haustorium has pushed into the central mass. From this preparation it would appear that the haustorium has pierced the plasma membrane and lies in the mucilage vacuole. In figure 59, however, where a section of a haustorium is shown lying between two masses of mucilage, the haustorium is completely surrounded by a clear area within the host cytoplasm. Moreover the cytoplasm with embedded starch grains can be traced completely around the masses of mucilage. The mucilage vacuoles are here unbroken, and the haustorium lies apparently in a space due to invagination. Invagination is more clearly shown in the case of mere indentation of a mucilage vacuole by a haustorium. Such cases are common in the sections. Figure 64 gives especially clear evidence for invagination. The section was stained with the Gram-Weigert stain. Both host walls and the heavy haustorial sheath are pink. All protoplasm is deep blue and the mucilage is yellow. A continuous film of the blue-stained host cytoplasm can be traced around the pink-walled haustorium, showing clearly between this and the yellow mucilage.

In the sections of leaves bearing teleutosori, storage starch is not in evidence. This disappearance is probably due both to the fungus and to the growth of the young host leaves. Free-hand sections of unrusted winter leaves, gathered when the young leaves of the plant were half grown, showed abundance of storage starch by the iodine test. This would indicate that the fungus is largely responsible for the disappearance of the starch in the infected leaves.

The haustorium is usually an unbranched inflated sac, but the tip is often recurved. A slender stalk shows in occasional sections (FIG. 60). The haustorium is typically binucleate (FIGS. 61, 62), corresponding to the binucleate condition of the mycelium. The nuclei lie in a granular cytoplasm which usually encloses a large vacuole at the tip of the haustorium (FIG. 62). Contacts between host nucleus and haustorium are rare. Haustorial sheaths are also less frequent than in the case of either *Puccinia Sorghi* or *Puccinia claytoniata*. The thickening shown around the base of the haustorium in figure 62 is stained blue with the Gram-Weigert stain, and so indicates layers of host cell cytoplasm rather than extensions of the host cell wall. Not infrequently instances are found of apparently dead haustoria with dense blue-stained cores encased in heavy pink-stained walls (FIGS. 63, 64). Figures 61 and 64 show cells just within the lower epidermis, and show the wide intercellular space between this layer and the epidermis, caused by the network of hyphae from which the sori arise.

GENERAL DISCUSSION

It becomes apparent through examination of rust-infected tissues that, important as the haustorium undoubtedly is for the life of the fungus, the fungus is not entirely dependent upon this organ for its nutrition. The early infection stages of the corn rust show an abundance of intercellular mycelium and well developed runners with dense contents. This would indicate that during the early stages, before the development of haustoria, the fungus is able to draw some nourishment from the host directly through the host cell walls. Certainly the vigorous intercellular growth shown in plate 1 does not depend upon haustorial activity.

I have observed the same condition in *Chrysomyxa Pyrolae* upon *Pyrola americana*. The mycelium apparently overwinters in buds or rootstocks. Leaf material examined in April shortly before the formation of the uredosori shows an abundant mycelium in the mesophyll but few haustoria. After the sori have become erumpent, haustoria are much more numerous. W. Magnus (1900) reports for *Puccinia leucospermum* on *Anemone nemorosa* that the haustoria are relatively late in appearing, and he thinks the chief nutrition of the fungus during the formation

of the spermogonial and aecidial anlagen must have been by way of the intercellular hyphae.

The rusts in such stages suggest in their host relations the thread blight of tea and *Acanthostigma parasitica*, which are described by Butler (1918). The thread blight of tea, a possible *Rhizoctonia*, abundant in India, spreads web-like films of sterile hyphae fan-wise on the under surfaces of the leaves. It causes a browning of the leaves, although the host cells are unbroken and the glistening strands of mycelium can be readily peeled off. *Acanthostigma parasitica* upon the pine has rudimentary haustoria. They are tiny outgrowths of the basal layer of the cushion-like masses of mycelium on the surface of pine needles and pierce merely the cuticle of the host cells. In both cases, Butler points out, the food passes through unbroken walls. The efficiency of such absorption methods is more comprehensible under Hansteen-Cranner's theory of the composition of the cell wall (1922). If the cellulose wall contains a network of the same proteins as the plasma membrane, then the two protoplasms will be in contact even by the mere external apposition of fungus and host cell walls.

Such data as the above do not minimize the importance of haustoria. They do, however, shift the emphasis in interpreting function. The mature haustorium effects a great increase of surface for absorption. Moreover, for the unencased haustorium at least, the absorption would be through fewer layers than in the case of the intercellular hyphae, since the haustorium penetrates the cell wall.

I have not observed in the corn rust any mucilaginous secretion under the appressorium, such as is reported by Brown (1915), Dey (1919), Boyle (1921), and Waterhouse (1921) as present under the tips of the penetrating hyphae of *Botrytis cinerea*, *Colletotrichum Lindemuthianum*, *Sclerotinia Libertiana*, and the germ tubes of the sporidia of *Puccinia graminis*. Although the resistance to displacement gained by such an attachment might possibly assist the act of penetration, Blackman considers it unnecessary. He has (1924) suggested that the essential preliminary to penetration is merely close adhesion of the tip of the hypha to the surface to be penetrated. He also holds that the adhering surface is so large, in comparison with a cross section of the peg-like penetrating projection, that but little pressure is

needed to resist the back pressure resulting from the outgrowth of the infection tube and to push the projection through the wall.

Penetration by means of a slender filament, which is so characteristic of the haustoria of *Puccinia Sorghi* and other rusts, is, in its lack of digestive action, different from the process of penetration in the case of the facultative parasites. In the latter, according to Brown (1915) and Blackman and Welsford (1916), working with *Botrytis cinerea*, Dey (1919), working with *Colletotrichum Lindemuthianum*, and Boyle (1921), working with *Sclerotinia Libertiana*, although the cuticle is penetrated by pressure alone, enzymic secretions of the hyphae soften the cell tissues. *Rhizopus nigricans* has been shown by Stevens and Hawkins (1917) to secrete an enzyme which disintegrates cellular tissue by digestion of the middle lamella, and a toxin which, by killing the plasma membrane, allows leakage of the cell contents. These examples indicate a specialization of offensive metabolism by the parasite, rather than an evolution toward adaptation to the living host.

Lutman (1910) traces the origin of the haustorium in *Entyloma Nymphaeae* through two stages. First, he says, a hypha is pushed out against a cell wall, and there branches into a closely appressed appressorium. Under this the host cell wall becomes dissolved and a hypha is pushed down from the appressorium into the cell. The pentration hypha is much constricted, but soon swells into normal diameter and branches profusely into a tangled knot. Lutman supposes that perhaps originally appressoria alone were produced, and that the penetrating hypha is a further development.

An evolution of penetration is suggested by Allen (1926) for the Uredineae. In her study of rust on the resistant Khapli emmer she detects a toxic effect of the appresoria upon the guard cells of the stomata. Inasmuch as such a secretion would not assist the fungus through the stoma, she suggests that 'the appresorial secretion is a survival from an earlier period in the evolution of the fungus when it did secrete enough to dissolve its way into the host.' She found no such secretion in connection with the haustorial penetrations. In her description of penetration into a susceptible host (1923) she mentions a red-stained spot on the host cell wall, which she believes indicates an alteration in wall composition due to enzymes secreted by the fungus. This is

at the spot where the filament develops. Since the rest of the wall under the appressorium is unchanged, she thinks the appressorium less an organ of attachment than a storage reservoir, from which the cell contents can be transferred with the greatest economy to the nearest point of entrance into the host cell. I have not found evidence in the corn rust of any alteration in the host cell wall at the point of entrance. The corn rust illustrates the statement of Bolley (1906): '[They] are such perfect parasites that the hyphae pass directly through living cell walls of the host and make practically perfect fusions with the cell-walls.'

That in young stages a hyaline zone intervenes between the haustorium boundary and the plasma membrane of the host cell is shown in my figures of plate 1. Apparently this later becomes the digestion cavity. That the haustorium invaginates rather than penetrates the cytoplasm seems clear from figures 29, 38, and 64. There seems to be general agreement on this point in the literature. However, we need clearer evidence as to whether each branch of a digitate haustorium has a special sheath of host cytoplasm, or whether the whole complex lies in a single rounded indentation of the host cytoplasm. This latter seems to be the case, for the branched haustoria of *Erysiphe graminis* (see fig. 19 by G. Smith, 1900). Leitgeb (1881) gives a graphic description of the cytoplasm of the fern prothallial cell streaming around the haustorium-like processes of *Completoria complens* and carrying the plastids in its stream. Such streaming is supposed to leave the plasma membrane immobile. By the establishment of haustorial lobes within folds of an invaginated plasma membrane, a condition for effective absorption is attained. The failure to rupture the primordial utricle is, I believe, the mark of an adapted parasite, an evidence of an approach to mutualism in the haustorial parasite, with its immediately destructive action reduced to a minimum.

Elaboration of form in the haustorium is another evidence of a high degree of adaptation by the haustorial parasite in securing maximum contact relations with the host protoplasm. The varied forms reported from the literature and from my own investigations, which I have grouped under Arnaud's four types, are merely elaborations of the oblong sac. The delicacy of the adjustment in this matter is particularly striking in the case of variation of form within one host. The characteristic haustorium

of the corn rust in parenchyma cells is the two- to several-lobed, digitate form which I have so often figured. In the epidermal cells, on the other hand, one finds more often simple sacs or reduced button-like forms. The many variations in form described in the literature indicate that haustorial form is a physiological as well as a morphological reaction.

The multinucleate condition so frequent in haustoria is another evidence of plasticity in the organ. Although my tabulation from the literature shows that in general haustoria have a single nucleus or paired nuclei, according as they arise from a gametophytic or sporophytic mycelium, the number is larger in the lobed forms.

Data upon the space relations of the haustorium and the host protoplast most frequently deal with the contact between the haustorium and the host nucleus. This emphasis upon the nucleus may be due in part to the fact that it is generally regarded as a center for the metabolic activities of the cell. There are interesting statements in the literature as to the persistence of the nucleus in infected cells. Magnus (1900) reports that in infected tissue the host cell nucleus often long outlives the rest of the protoplasm. Osner (1910) reports that in the sori of Timothy smut the host cell nucleus is the last thing to disappear before spore formation. Arzberger (1910) writes of the mycorrhiza in *Eleagnus argentea*, 'Thus there is a long continued struggle between the host and the fungus resulting finally in the destruction of the cell, the nucleus being its most resistant part.'

The evidence in the literature appears to favor the view that there is an attraction between host nucleus and haustorium. I have listed twenty-three cases of habitual contact, against nine cases of occasional contacts, and three negative cases. In view of the numerous positive findings, these negative cases are perhaps to be regarded as due to special and exceptional conditions.

The interest in these data as to contact relations between haustorium and host nucleus centers in the bearing they have on problems of the physiological relations within the cell. Statements as to the initiation of the contacts fall under two heads: first, contacts due to host-nuclear control; second, contacts due to reaction by the haustorium. The assumed action of the host nucleus it is thought may be protective. Guttenberg (1905) in

the case of *Ustilago Maydis*, interprets the contact of host nucleus and haustorium as a means of initiating the building of a cellulose sheath around the haustorium by the host cell. Colley (1918) also believes that the cellulose sheath which he finds around the haustoria of *Cronartium ribicola* is a means of defense by the host cell, but he finds little evidence that the nucleus is in any way concerned.

The action of the host nucleus may be offensive. Magnus (1900) ascribes to the influence of the host nucleus the reduction of the haustoria of *Puccinia Xanthii* to formless masses. It is the explanation given by Němec (1911) for the relations of host nucleus and haustorium in *Uromyces Betae* on the beet. Němec thinks that the haustorium is drawn into contact by the host nucleus and that, through this contact, degeneration and death of the haustorium ensue. Němec calls this nuclear action a temporary phagocytic activity of the host. At other times he finds the nucleus ineffective in its offense when a new branch may be put out by the haustorium below the degenerate tip. One of the phenomena which Allen (1926) describes in the relations of *Puccinia graminis Tritici* and the resistant host, Khapli emmer, is a host-nuclear offensive, initiated, she thinks, as a chemotactic response to substances diffused out into the host cell by the haustorium. The effect on the fungus she ascribes to secretion from the host.

The second explanation of contacts, as due to reaction by the haustorium, assumes usually a positive tropism on the part of the haustorium, to further absorption. Thus Sappin-Trouffy (1896) regards the contact of haustorium and host nucleus as a means by which the parasite draws to itself the products of the host cell metabolism. Guttenberg (1905) states that the haustoria of *Puccinia Adoxae* grow toward the host nucleus in order to absorb nuclear substances. He finds a similar tendency in the case of the haustoria of *Uromyces Alchemillae* in leaf tissue, but finds no contacts in the cells of petioles. Such an exception makes it appear that we are still far from any complete explanation of the relation of haustorium and host nucleus.

The growth of the haustorium toward the nucleus is rarely interpreted as an attack. Zach (1910), however, so describes infection by *Puccinia graminis*. He shows elaborate figures of a branched haustorium making a tangled network within the host

cell nucleus. Both invader and nucleus, he states, are finally killed, and the infected hyphal-filled nucleus becomes a homogeneous, amorphous excretion body, with degenerate hyphae projecting from its surface as papillae. The figures suggest an intracellular hyphal fungus rather than haustoria.

These same problems of the relations between host cell nucleus and parasite are presented by the intracellular mycorrhizas. A comparison is the more interesting because, according to reports, one finds in the mycorrhizal complex a change in relations during the life cycle of the fungus. This is shown by the work of Groom (1895) upon *Thismia Aseröe*, Magnus (1900) upon *Neottia Nidus-avis*, Arzberger (1910) upon *Ceanothus americanus*, *Eleagnus argentea*, and *Myrica cerifera*. In all these cases the authors report that the fungus in the early stage grows at the expense of the host but with a controlled mutualism. In all but *Neottia Nidus-avis* the hyphae grow directly toward the nucleus. As the masses of intracellular hyphae increase, the balanced condition ceases. In *Thismia Aseröe* and *Neottia Nidus-avis* the host cell nucleus gains control and the fungus degenerates into tangled masses of hyphae, the sporangioles, from which the host cell is believed to draw some nourishment. In the forms studied by Arzberger the host cell never gains this complete control, although the hypertrophy of its nucleus is taken to indicate activity. In all three forms the parasite draws increasingly upon the host until both cytoplasm and nucleus of the host cell are killed and more or less absorbed. After this outcome, the fungus also dies, probably, Arzberger says, of starvation.

In my investigations upon various rusts I have shown that haustorium and host cell nucleus are frequently in contact. The appearance of the nucleus, however, even when enwrapped by the haustorial lobes, does not suggest offensive action on either side. According to Arzberger (1910) for mycorrhiza, and according to Zender (1924) for the haustorial cell which pushes out from the absorption process of *Cuscuta*, hypertrophy and lobing of the nucleus indicate phagocytic activity. There seems little indication of this in the case of rust-infected cells. I have noted that the nuclei are occasionally hypertrophied, but this hypertrophy I should interpret, as Allen (1923) does in the wheat, as a stimulation effect found in a susceptible host. The haustoria

in these cases of contacts seem generally in active condition, as indicated by the density of their protoplasm. If there is special significance in the haustorium-nucleus contact, it seems to me more likely to be one involving absorption processes by the haustorium. We need clearer evidence as to the functions of the nucleus as a center of metabolic activities.

The peculiar, conspicuous, and highly stainable thickenings of, or sheaths about, the haustorial walls provide perhaps the most direct and at the same time the least understood evidence of the nutritive relations between the rusts and their hosts. That these structures are sheaths deposited by the host cell is perhaps the most generally accepted view, and in the case of the mildews it is clear that the sheath of the stalk is continuous with the cellulose wall of the host cell. In the case of the haustoria of corn rust, however, in many cases the ring and cup-shaped structure seem to be indistinguishable from a thickening of the wall of the haustorium. Such sheaths or thickenings, according to reports which I have listed from the literature, are certainly of wide spread occurrence. In these reports they are generally held to be an evidence of resistance by the host to the parasitic attack.

There is a marked difference in the time of their origin. In *Erysiphe communis*, as described by G. Smith (1900), their origin is very early. At first a thickening of the host cell wall keeps pace with the penetrating haustorium. Enzymic action by the growing haustorium gradually dissolves this, leaving the mature haustorium surrounded by a turgid digestion sac, and sheathed at the base by undissolved host cellulose. Colley (1918) reports a later origin for the sheath in *Cronartium ribicola*. He states that the sheath has not been found in connection with young haustoria in any case, but that proper staining shows around the mature haustorium a sheath, which is thick at its base and tip, and very thin in the middle region. Colley explains this peculiarity of structure as the result of response by the host at the two chief points of irritation, 'the point in the cell wall through which the haustorium entered, and the point of contact of the advancing haustorial tip with the host cytoplasm.' I think the objection may be raised here that any response to the irritation at the spot of penetration of the haustorial neck would naturally come early, when the projection filament is first piercing the host cell wall. Colley also suggests that the sheathing cup and cap are precipi-

tation products laid down as the result of irritation in the narrow space between the haustorium and the membrane of the host cytoplasm. Allen (1923) describes a sheath for *Puccinia graminis Tritici* only in the case of extreme incompatibility between *Puccinia graminis Tritici*, form 3, on Mindum wheat. She interprets it as a cause of death to the haustorium. She notes the frequent massing of host cytoplasm around dying haustoria, and thinks this may have some relation to sheath formation. The sheath, she states, quickly loses its power to stain.

Many workers upon rusts do not report a sheath. Ward (1902b) does not describe one for *Uredo dispersa*, nor Evans (1907) for *Puccinia Sorghi*. If the sheath is a result of resistance offered by the host, its absence in certain rust species may be explained as indicative of well established mutualism in the host-fungus complex. Interpretations as to the significance of the sheath should be made only after a comparison of haustoria through the full life cycle of the rust and under varying host conditions. In my investigations upon various rusts, although I have observed some degree of sheathing in the majority of cases, I have obtained data for comparison only in the case of the corn rust. It seems to me that we have in the degree of development of the sheath or thickening around the corn rust haustorium an index of the degree of mutualism effected at the moment by the fungus and its host. In the early stages of the rust growth, including the penetration and development of the lobed haustoria, the absorption by the fungus is so adjusted that little reaction is set up by the host, and a sheath is rarely formed. In later stages the drain upon the host, evidenced externally by the yellowness of the infected areas, may stimulate the host to deposits of cellulose. Capacity for sheath formation would thus become a factor in the expression of immunity. It will be of importance to test this theory by testing the relative immunity of a large number of races of corn and at the same time the relative development of haustorial sheaths in the various combinations.

The effectiveness of a host cellulose deposit as a means of preventing absorption by the haustorium is so far untested. If cellulose walls allow the passage of food through them into intercellular hyphae, then cellulose sheaths can at best merely lessen the amount absorbed. A further point for study is the chemical nature of the so-called sheath. Chitin has been reported as oc-

curring in the walls of many fungi. Wisselingh (1898) reports its occurrence in the Erysipheae (reported by G. Smith, 1900). Smith confirms Wisselingh's negative test with Congo-red as evidence for the occurrence of chitin in the Erysipheae, and adds:

> The walls of the haustoria do not show any reactions to stains that would indicate that they contain any different material from the remainder of the fungus.

So far as I find, the haustorial thickenings of the Uredineae have never been tested for chitin.

An indication of the effectiveness of the sheath in checking absorption may be gained from the condition of the enclosed haustorium. Colley traces in the haustorium a vacuolization of the cytoplasm, and a shrinking of the nucleus coincident with the sheath development. He states that this lessens the efficiency of the haustorium as an absorbing organ, but he does not say what he thinks is the direct cause of the degeneration. Allen, as mentioned above, connects the sheath with the death of the haustorium. The development of the sheath does not seem to bring degeneration to the haustorium of the corn rust. It is true the haustoria of the corn rust are seldom completely encysted, but when they are, a median optical section usually shows the nuclei of the haustorium still present (FIG. 39, PLATE 4). Occasionally the contents of the haustorium are reduced to a narrow strand (FIG. 38). The continued vitality of the enclosed haustorium is apparent in the many cases where non-sheathed lobes project from a heavily sheathed base (FIG. 39).

My study of many stages of rust-infected corn tissue, and of material from various other rusted plants, makes apparent the prevailingly normal condition of the plastids in infected cells. In their position and size there is little difference between the plastids of infected and uninfected cells. The lessened staining capacity observed in plastids of infected cells is not necessarily due to a toxic effect. Mains (1917) has suggested that the feeding fungus checks normal storage. Possibly this lessened staining capacity of the plastid is due to the same cause as that which Meyer (1918) demonstrates for the normal aging leaf, a drawing upon protein of the plastid for respiration, under shortage of carbohydrates.

I find reported eight cases of disorganization in the plastids of rusted tissue. Such cases as the coffee rust and asparagus rust

seem to be the result of toxic action on the part of the rust, though they require further study.

The 'green island' phenomenon of infected tissue is strongly correlated with specialization in the parasite. As the literature shows, thirteen of the nineteen examples of chlorophyll persistent in infected tissue longer than in surrounding non-infected tissue are due to rusts. One of the other six is due to a haustorial fungus, *Uncinula Aceris*. The basic fact here is, of course, the prolongation of the life of the entire cell, and not merely the persistence of the green color. The stimulation of infected cells to a longer and possibly more active life than the adjoining cells is one of the least understood phenomena of parasitism. As a physiological effect it is perhaps to be classed as a less specialized case of those mycetogenous deformations and new formations which DeBary (1887) holds are 'in direct causal relations with the feeding of the fungus.' The difference between these 'green islands' and the gall formation is from this standpoint merely one of degree.

In the instances of 'green islands' which I have listed from the literature there is little discussion of their cause or of the relation of the phenomenon to resistance and susceptibility. The reference from Eriksson and Henning (1896) is merely to two colored figures which show the green spots vaguely. They are not discussed in the text, which is concerned with the relation of teleuto- to uredospores in the aging leaves figured. The series of colored figures of rusted oat leaves, however, does show clearly the contrast between the shift from pale green borders to yellow borders around sori on green leaves, and green borders around sori on the old, reddened leaf. The reference from Carleton (1899) is also to a colored figure. It shows very clearly the green-bordered sori on a red, basal, rye leaf, but Carleton's description of the figure notes merely that 'the chlorophyll remains about rust spots during changes of the coloring matter.' Mains (1917), the first one in my list to give any detailed consideration to the persistent green spots, interprets them as evidence that the infected cells levy upon the uninfected cells surrounding them. 'It would thus appear that the rust thus destroys the symbiotic balance between the cells of the host and causes some of them to have parasitic relations with the rest.' This interpretation does not touch the cause of the phenomenon, but Mains in his descrip-

tion of infection experiments with *Puccinia Sorghi* notes that the green spots appear 'when limited areas of the leaf are infected.' He calls attention to them in the cases of abortive infection on corn plants which were subjected after inoculation to unfavorable degrees of temperature or moisture, or to air lacking carbon-dioxide.

I think the fact should be more strongly emphasized that the 'green island' is most strikingly developed when causes other than the fungous infection have started deterioration in the surrounding tissue. Consideration of the subnormal condition of the adjacent host tissues should, it seems to me, be the starting point for any discussion of the physiological problems involved in the 'green island' phenomena. In the normal course of the infection of a vigorous host, any microscopic evidence of a symbiotic adjustment between host and parasite is often obscured by the heavy drain from the host by the parasite, but when other agencies tend to inhibit host development, the delicate adjustment between parasite and host becomes especially evident, and one may find the fungus effecting a preservative action upon the infected area. The water-soaked corn leaf which I described is an illustration. In this case the death of the surrounding tissue was not due to the infection.

In several of the cases listed from the literature the inhibiting factor upon the host may be inferred from the descriptions. Upon this basis they may be re-arranged as follows:

Factors inhibiting spreading infection in host tissue

1. Lack of water		
(a) Due to aging of the leaves		
Eriksson and Henning,	*Puccinia coronata,*	*Avena*
Carleton,	*Uromyces rubigo-vera secalis*	Rye seedling
Thomas,	*Septoria Apii*	Celery
(b) Due to injury		
Galloway,	*Coleosporium Pini*	*Pinus*
(c) Due to reduction in water supply		
Mains,	*Puccinia Sorghi*	Corn
2. Lack of carbon-dioxide		
Mains,	*Puccinia Sorghi*	Corn
3. High temperature		
Mains,	*Puccinia Sorghi*	Corn
4. Reduction of light		
Long,	*Puccinia graminis*	Wheat
Long,	*Puccinia coronata*	Wheat

5. Hypersensitiveness

Stakman,	*Puccinia graminis Secalis*	Rye
Fromme and Wingard,	*Uromyces appendiculatus*	Bean
Allen,	*Puccinia graminis Tritici*	Khapli emmer

Allen does not note the 'green island' occurrence in a susceptible host, but she notes its frequent occurrence in the two less resistant forms of Khapli emmer. She notes it under inhibiting conditions of host growth, when 'the infected leaf dies prematurely.' She also interprets the 'green island' as a sign of balanced achievement in a resistant host, describing an actual change in the plastids as the result of the presence of the fungus. She states that the plastids, after shrinking and so causing the initial pale fleck which indicates lack of adjustment between resistant host and toxic pathogene, regain their normal size in the central cells of the infected spot, and cause a persistent green spot in the midst of impoverished or collapsed cells. Allen sees in the whole reaction less a question of food supply than of secretions, which finally stimulate instead of poison.

This recovered condition of the infected cells corresponds apparently to the balanced condition between rust and host cells in a susceptible host. Reduced virulence during what may be compared to the incubation period in the case of certain bacterial parasites is known among both smuts and rusts. Thus a smut, until the time of sporulation, is quite innocuous to its green host. Among the rusts such lessened virulence is even more striking in certain species which have a perennial mycelium. Such a condition is described by Hartig (1882) for *Melampsora Geoppertiana* upon the cowberry. During its first year the mycelium spreads through the cortex without any stimulation effects. Only in the second year are the long quill-like hypertrophies formed, upon which teleutospores develop. Kursanov (1917) cites the prolonged dormant period of *Melampsora Pyrolae* as a case of reduced virulence. He thinks it overwinters in the terminal bud, but it does not make itself evident in the leaves developed from this bud until the second spring. I have made observations upon this rust, which recurs annually in a plot of *Pyrola americana* at Norton, Massachusetts. Both in the fall and in the spring before the rust's appearance in the leaves, I have found a healthy, although scanty, intercellular mycelium in bud scales and rootstock.

That there is actually a mutualistic relation between rust parasite and host is of course hardly conceivable. However, the relations between the cells of host and parasite, as I have observed them in various rusts, are indicative of reduced or entire absence of toxic action on the part of the parasite. Its cells relate themselves to those of the host as do its own heterotrophic and storage tissues. Even wheat rust, perhaps the most destructive parasite which we have, is highly specialized in its reduced toxic effect on the host. Allen (1923) writes of a seven-day infection in susceptible Baart wheat, 'Here, as in the younger material the host cell gives every evidence of functioning normally or even with somewhat heightened activity, and host and parasite seem fully congenial.' There are cases of hyperparasitism among the rusts. Stevens and Hall (1910) lay the slight economic importance of *Puccinia Sorghi* to the fact that it does not usually develop early in the season. My cytological studies point to reduced toxicity and controlled feeding as added reasons for the lesser injury done by this parasite. I think there are indications of some such control in the case of the other rusts studied. *Claytonia* plants infected with *Puccinia claytoniata*, and violet plants with *Puccinia Violae*, blossom abundantly, notwithstanding rust-spotted leaves. *Houstonia* infected with *Uromyces houstoniatum* becomes so etiolated that the yellow, slender plants are easily detected in the field, but the plants are not killed; they blossom and fruit even with rusted calyx. *Aecidium punctatum* causes sterility in *Hepatica acutiloba*, but the rusted leaves, although metamorphosed, are green and vigorous. In the plot of *Arisaema* infected with *Uromyces Caladii*, which has been under my observation for four years, even when spermogonia and aecidia are abundantly developed, the leaves are not discolored; in September ripe fruit and a few persistent leaves show that the plants carry out their full cycle. The climax of hyperparasitism among the rusts is found in *Chrysomyxa Pyrolae*. Whereas *Puccinia graminis* infects the young wheat plant and, in coming to maturity, impoverishes the maturing host, *Chrysomyxa Pyrolae* appears on the leaves of *Pyrola* at the end of their career in the spring. In the plot of infected *Pyrola americana* which I have observed for four successive years, the layer of uredosori and teleutosori so thickly covers the under surfaces of the leaves that their brilliant orange is striking in the undergrowth of the grove. The entire lower

epidermis is torn away by the erumpent sori, but the leaves show little discoloration. The excessive sporulation of the fungus does not prevent the new leaves of the host from developing normally and making a healthy plot of large shining leaves each year. I have also found fruited stalks in the fall. Kursanov (1917) considers that either the *Pyrola* leaf, or the mycelium, or both, must mature in some way before the conditions necessary for the fruiting of the rust are attained. He considers that the extraordinary length of this maturation process is a distinctive characteristic of this rust.

The cases which I have cited indicate that these rusts are all so hyperspecialized, even among the specialized rusts, that they do little damage to the host except at the time of spore production. For the Uredineae in general, I think that an elaborate development of the haustorium has furthered the lack of disturbance in the regions of special metabolism of the host-parasite complex.

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Description of plates

The figures of PLATES 1 to 9, with the exception of FIG. 1, were drawn with the aid of a camera lucida from sections 5–7.5 microns thick. A Leitz microscope was used with a 1/16 inch oil immersion objective and an ocular 2. Unless otherwise stated, the material was fixed in Flemming's medium solution, and stained in Flemming's triple stain. FIG. 1 was drawn with the aid of a camera lucida from a strip of epidermis with Leitz objective 7 and ocular 3. FIG. 1 is magnified approximately 750 times; the other figures, approximately 1200 times. The figures of PLATES 8 and 9 are free-hand drawings made from living plants; they were drawn actual size, but have been reduced in reproduction.

PLATE 1

Early stages of infection of corn tissue with Puccinia Sorghi

FIG. 1. A germ tube of *Puccinia Sorghi* at a stoma. The preparation was mounted in lacto-phenol and stained with Coton Bleu.

FIG. 2. Intercellular hyphae and an appressorium containing two nuclei.

FIG. 3. Haustorium mother cell in contact with an epidermal cell sending papilla into the cell.

FIG. 4. Slightly later stage of a penetration filament. a. View at lower focal plane. b. View at upper focal plane.

FIG. 5. Portion of an epidermal cell showing young haustorium and mother cell.

FIG. 6. Young haustorium showing a deeply stained center and two deeply stained granules in the stalk.

FIG. 7. Young haustorium showing the beginning of lobing in terminal knob.

FIGS. 8, 9. Later stages of lobed haustoria. (The stalk in figure 9 should be as distinct as that in figure 8.)

FIG. 10. A lobed haustorium in an epidermal cell arising from a branched hypha.

FIG. 11. A haustorium in contact with the nucleus of a mesophyll cell. The conjugate nuclei of the haustorium mother cell are clearly defined.

FIG. 12. A lobed haustorium in contact with the host cell nucleus of a mesophyll cell.

FIG. 13. A mature lobed haustorium in a mesophyll cell, showing conjugate nuclei in the lobe which indents the host cell nucleus.

PLATE 2

Puccinia Sorghi

FIGS. 14–18. The material was fixed in Carnoy's solution and stained with Flemming's triple stain. The section was cut longitudinally.

FIG. 14. Two mesophyll cells, each containing a lobed haustorium with slightly thickened stalks.

FIGS. 15, 16. Mesophyll cells containing haustoria showing variations in stalk development.

FIG. 17. Portion of an epidermal cell showing a haustorium with a thickening halfway up the slender stalk. (What appears to be a dash at the tip of the haustorium was not present in the original drawing.)

FIG. 18. Portion of a mesophyll cell containing a haustorium with a thickened stalk.

FIGS. 19, 20. Older stages of the rust taken from a green leaf bearing erumpent sori lying in yellow areas.

FIGS. 21–23. Cells from tangential section of 'green island' tissue, fixed in 50 per cent alcohol.

PLATE 3

Puccinia Sorghi

Late stages of infection from leaves with erumpent sori surrounded by yellow spots in a green leaf.

FIGS. 24, 25. Lobed haustoria arising from thickened bases and extending in direction of the long axis of the cell.

FIGS. 26–29. Haustoria in epidermal cells showing various degrees of sheathing.

FIG. 30. A mesophyll cell showing two haustoria with elongated lobes arising from thickened stalks.

FIG. 31. A cell just below a uredosorus containing two haustoria arising from spore bearing hyphae.

FIGS. 32–34. Cells showing heavy-walled haustoria from leaves of Golden Bantam corn.

PLATE 4

Puccinia Sorghi

FIG. 35. Hypertrophied, infected cell from a green leaf sheath near a uredosorus.

FIG. 36. An uninfected cell from the same tissue.

FIG. 37. A thick-walled haustorium in a thick-walled cell from the parenchyma tissue of a young corn cob.

FIG. 38. An encased haustorium from a thick-walled cell near a teleutosorus on a leaf sheath.

FIG. 39. Hypertrophied infected cell from a yellowed leaf sheath near a teleutosorus.

FIG. 40. Portion of an infected parenchyma cell near a teleutosorus upon the midrib of a leaf.

PLATE 5

Puccinia Sorghi

Cells from a leaf of outdoor corn, fixed in 50 per cent alcohol.

FIGS. 41–43. Mesophyll cells near the green edge of leaf, showing heavy infection but little sheathing of haustoria. (Figure 44, right, center, should be figure 42.)

FIGS. 44–47. Mesophyll cells from the yellowed area of the section, showing haustoria with various degrees of sheathing.

PLATE 6

Puccinia claytoniata, P. Violae, Aecidium punctatum, Puccinia asterum, Uromyces appendiculatus, Uromyces houstoniatus, Uromyces caladii

FIGS. 48, 49. Portions of cells from leaf of *Claytonia virginiana* infected with *Puccinia claytoniata.*

FIGS. 50, 51. Cells of *Viola cucullata* infected with *Puccinia Violae.*

FIG. 52. Cell from a leaf of *Hepatica acutiloba* infected with *Aecidium punctatum.*

FIG. 53. Cell from a leaf of *Solidago* sp. infected with *Puccinia asterum.*

FIG. 54. Portion of a cell from leaf of Kentucky Wonder Bean infected with *Uromyces appendiculatus.*

FIG. 55. Portion of a cell from leaf of *Houstonia caerulea* infected with *Uromyces houstoniatus.*

FIG. 56. A cell just beneath the epidermis of a spadix of *Arisaema triphyllum* which bore erumpent spermogonia of *Uromyces Caladii.*

PLATE 7

Aecidium Sambuci, Chrysomyxa Pyrolae

FIGS. 57. 58. Cells from a leaf gall of *Sambucus canadensis* infected with *Aecidium Sambuci.*

FIGS. 59, 60. Cells from a uredo-bearing leaf of *Pyrola americana* infected with *Chrysomyxa Pyrolae.*

FIGS. 61–64. Cells from teteuto-bearing leaves of *Pyrola americana* infected with *Chrysomyxa Pyrolae.*

PLATES 8 AND 9

Puccinia Sorghi

Leaves exhibiting typical stages of infection with *Puccinia Sorghi* found in a field of Golden Bantam corn; they show the color effects of infected areas of leaves of varying age and varying degrees of infection.

FIG. 65. Pale green infection areas; a moderate infection in a vigorous leaf.

FIG. 66. Dead infection areas; a heavy infection in a vigorous leaf.

FIG. 67. Green infection areas in a dying leaf.

FIGS. 68–70. Green infection areas in dry basal leaves.

5
1
2
6
4a
3
7
4b
8
10
13
9
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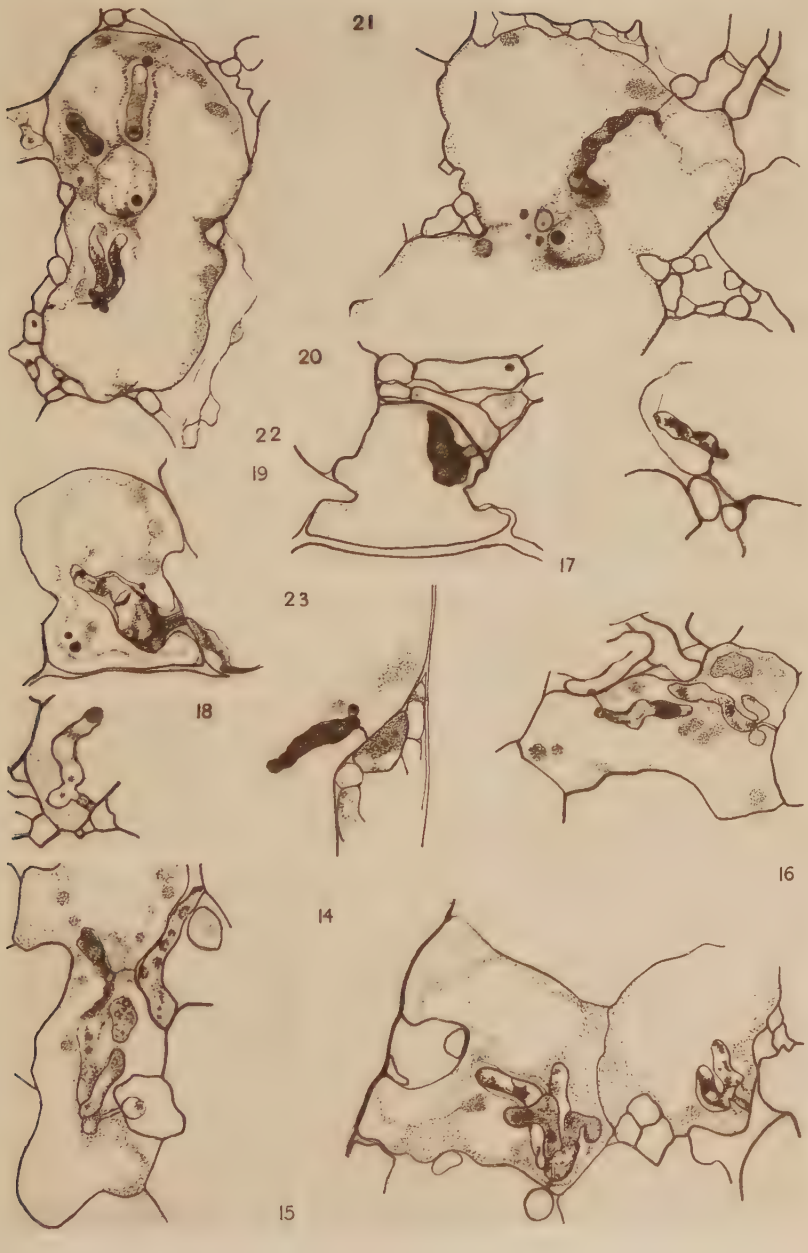
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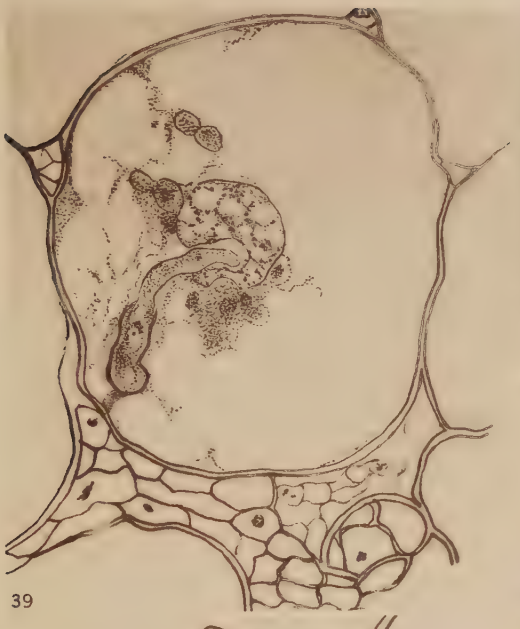

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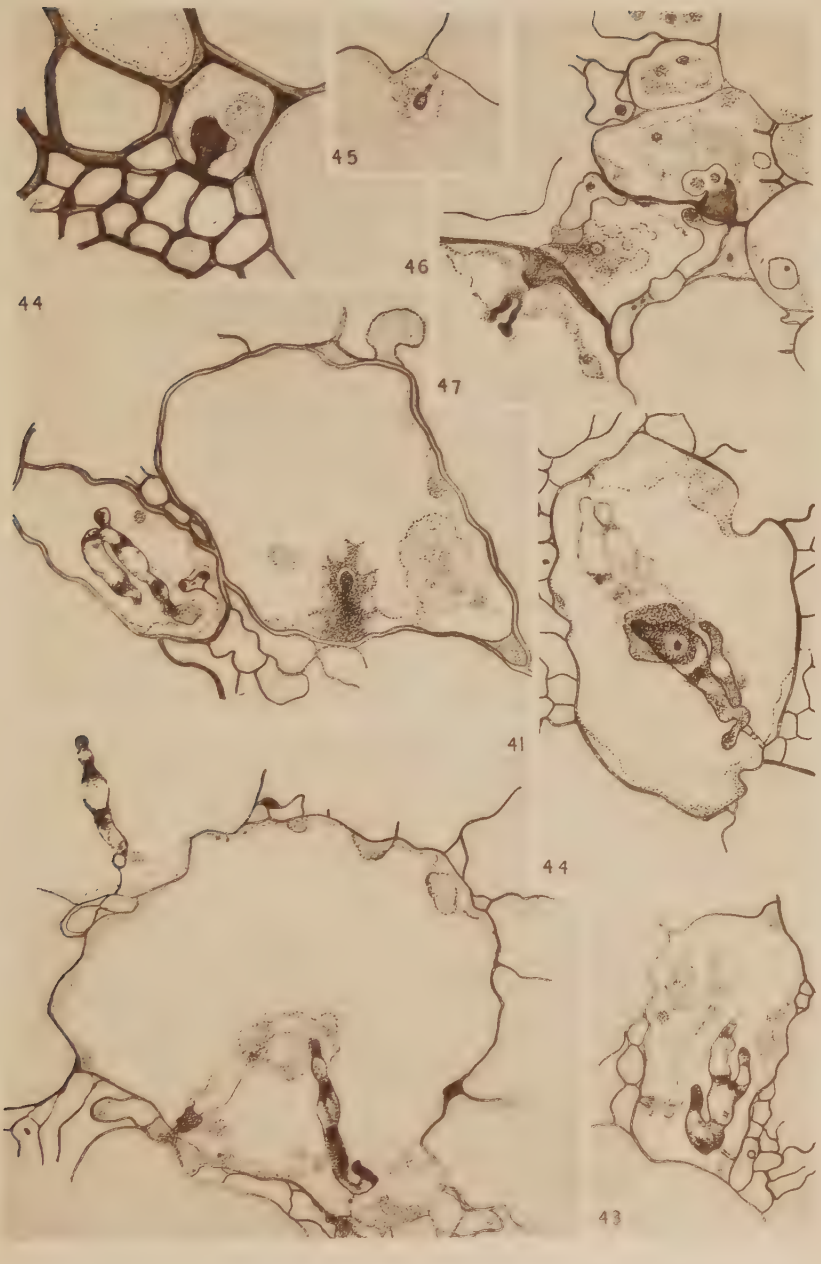
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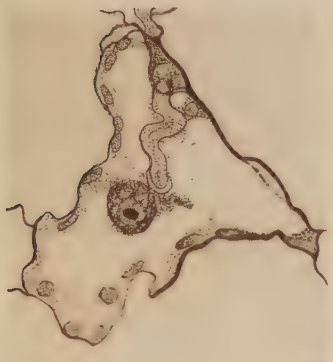

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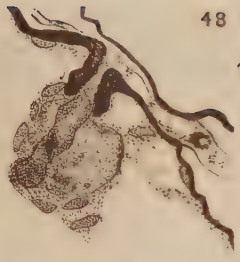

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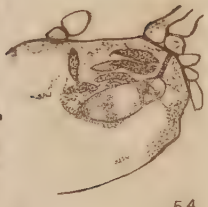

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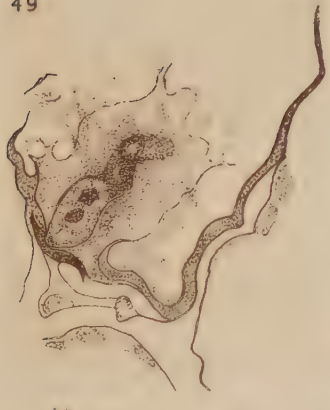

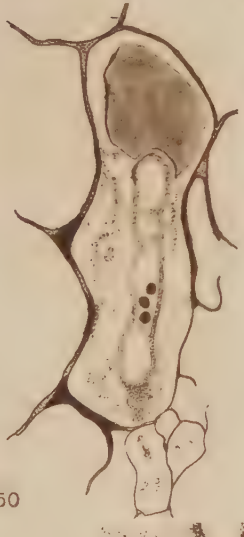

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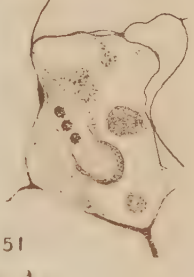

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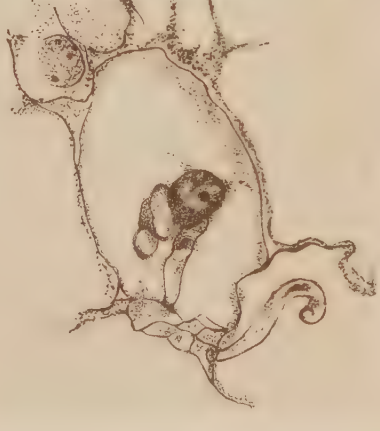

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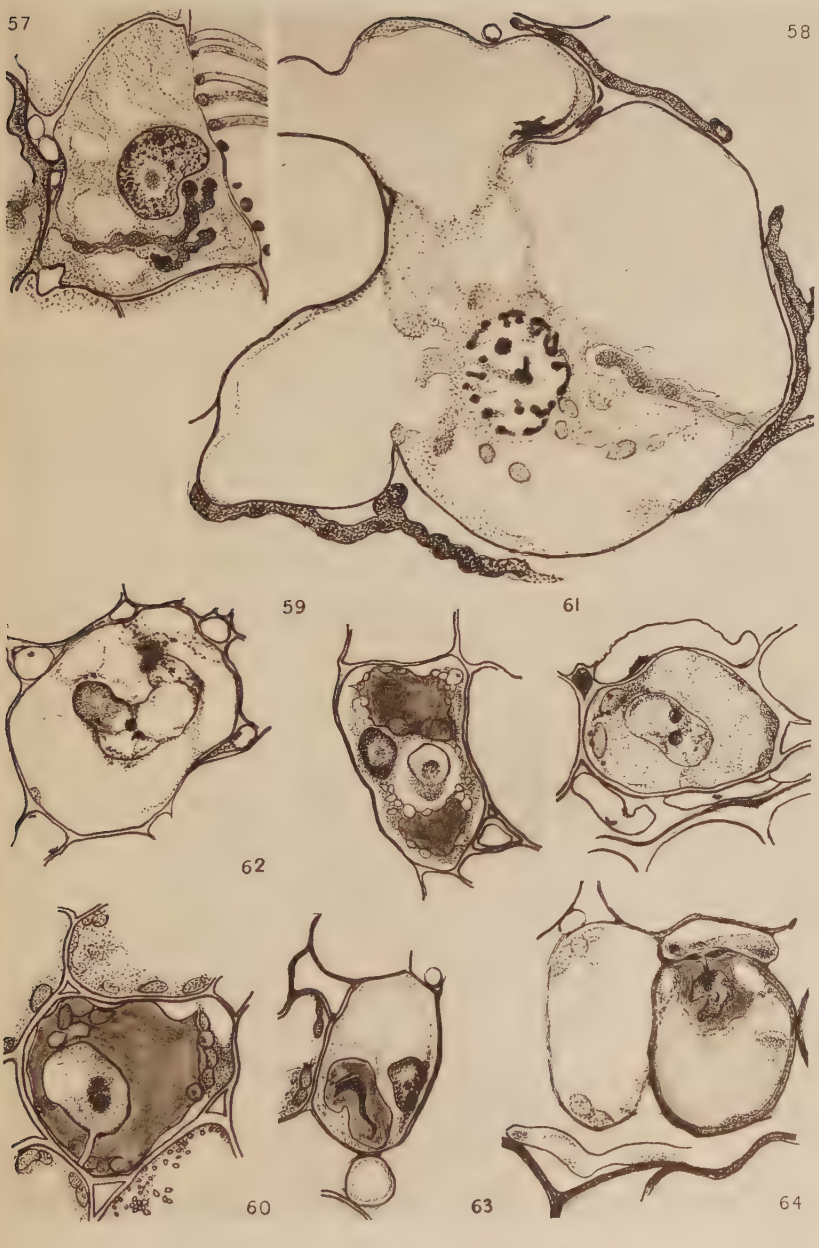
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FIG. 65. FIG. 66. FIG. 67.

PUCCINIA SORGHI ON GOLDEN BANTAM CORN

FIG. 68. FIG. 70. FIG. 69.

PUCCINIA SORGHI ON GOLDEN BANTAM CORN

VITA

Mabel Agnes Rice was born in Pittsfield, Massachusetts, November 14, 1875.

She attended the following educational institutions: High School, Pittsfield, Massachusetts; Smith College, Northampton, Massachusetts; and Columbia University, New York. She received the degree of bachelor of arts from Smith College in 1898, and the degree of master of arts from Columbia University in 1921.

Her educational record includes courses in plant physiology under Professor W. F. Ganong, of Smith College, and under Professor H. M. Richards, of Barnard College; bacteriology under Professor Jean Broadhurst, Teachers College; immunity under Professor Hans Zinsser, Columbia University; morphology under Professor C. C. Curtis, Columbia University; cytology, mycology, seminar, and research under Professor R. A. Harper, Columbia University.

She taught biology at the High School, Pittsfield, Massachusetts, from 1900 to 1920, was assistant in biology at Teachers College, Columbia University, from 1920 to 1922, and has been professor of botany at Wheaton College, Norton, Massachusetts, since 1922.

She published "Internal sori of *Puccinia Sorghi*" (Bull. Torrey Bot. Club **51**: 37–50. *f. 1–4.* 1924.).